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IL-15 independent maintenance of virus-specific CD8$^+$ T cells in the CNS during chronic infection

Jun Zuo [a,1], Stephen A. Stohlman [b], Gabriel I. Parra [b], Cornelia C. Bergmann [b,*]

[a] Department of Pathology, University of Southern California Keck School of Medicine, Los Angeles, CA 90033, United States
[b] Department of Neurosciences, Lerner Research Institute, Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, OH 44195, United States

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ABSTRACT

The role of IL-15 in T cell survival was examined during chronic CNS coronavirus infection. Similar numbers of virus-specific CD8$^+$ T cells were retained in the CNS of IL-15$^{-/-}$ and wt mice, consistent with loss of IL-2/15 receptor (CD122) expression. IL-15 deficiency also had no affect on IL-7 receptor (CD127) expression, Bcl-2 upregulation, granzyme B expression, or IFN-γ secretion in CNS persisting CD8$^+$ T cells. Furthermore, CD8$^+$ T cell division in the CNS was reduced compared to spleen. CD8$^+$ T cells in the persistently infected CNS are thus characterized by IL-15 independent, low level proliferation and an activated/memory phenotype.

1. Introduction

Memory CD8$^+$ T cells localized in both lymphoid and non-lymphoid tissues are important components of adaptive immunity, protecting against recurrent infection (Lefrancois and Masopust, 2002). IL-15 and IL-7 are important homeostatic cytokines for memory CD8$^+$ T cells in secondary lymphoid tissues (Surh et al., 2006). IL-15$^{-/-}$ mice have a profound defect in memory-type CD44^{Hi} CD122^{Hi} (IL-2/15 R β chain) CD8$^+$ T cells (Kennedy et al., 2000; Surh et al., 2006) and progressively loose memory CD8$^+$ T cells following acute infection (Becker et al., 2002). Nonetheless, a subset of IL-15-independent memory CD8$^+$ T cells survives following viral infections (Becker et al., 2002; Obar et al., 2004; Schluns et al., 2002; Zuo et al., 2005), potentially supported by IL-7 (Carrio et al., 2007; Surh et al., 2006). During viral persistence chronic or sporadic antigen (Ag) exposure may also contribute to maintenance of Ag experienced CD8$^+$ T cells (Obar et al., 2004). The regulation of CD8$^+$ T cell survival and function in non-lymphoid tissues, especially during chronic viral infections is poorly understood (Bergmann et al., 1999; Ely et al., 2006; Hawke et al., 1998; Khanna et al., 2003; Masopust et al., 2006; Zhou et al., 2004). In numerous models of virus-induced encephalitis CD8$^+$ T cells are prominent anti viral mediators, which are retained in the CNS for prolonged periods, even in the absence of detectable infectious virus (Griffin, 2003; Hawke et al., 1998; Ramakrishna et al., 2004; van der Most et al., 2003). Nevertheless, little is known about the maintenance of virus-specific CD8$^+$ T cells in the CNS, where the blood-brain-barrier (BBB) and limited MHC molecule expression on resident cells (Galea et al., 2007; Griffin, 2003) pose additional constraints on T cell homeostasis.

This report investigates the role of IL-15 in maintaining virus-specific CD8$^+$ T cells in the CNS during chronic infection by the neurotropic JHM strain of mouse hepatitis virus (JHMV). JHMV CNS infection is controlled within two weeks (Bergmann et al., 1999; Bergmann et al., 2006; Ramakrishna et al., 2004), yet viral RNA persists at very low levels (Marten et al., 2000). Despite a 50% reduction in the absolute numbers of virus-specific CD8$^+$ T cells in the CNS of IL-15$^{-/-}$ mice compared to wt mice during acute infection, the fraction of virus-specific cells was similar and immune control of infectious virus was not affected (Zuo et al., 2006). The present study demonstrates that IL-15 deficiency did not alter levels or effector functions of virus-specific CD8$^+$ T cells persisting in the chronically infected CNS. CD8$^+$ T cells retained within the CNS of both wt and IL-15$^{-/-}$ mice expressed low levels of CD122 and CD127 throughout persistence and only a small proportion of CNS persisting CD8$^+$ T cells were in cell cycle. These data suggest that the vast majority of virus-specific CD8$^+$ T cells maintained in the CNS are derived from acute effector cells and survive independent of IL-15.

Abbreviations: BBB, blood brain barrier; CLN, cervical lymph nodes; i.c., intracerebral; JHMV, JHM strain of mouse hepatitis virus; p.i., post infection; SC, spinal cord; T_{CM}, central memory T cells; T_{EM}, effector memory T cells.

* Corresponding author. 9500 Euclid Avenue NC30, Cleveland, OH 44195, United States. Tel.: +1 216 444 5922; fax: +1 216 444 7927.
E-mail address: bergmac@ccf.org (C.C. Bergmann).
[1] Present address: Department of Pediatrics, David Geffen School of Medicine at UCLA,10833 Le Conte Ave., 22-442 MDCC, Los Angeles, CA 90095, United States.

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2. Materials and methods

2.1. Mice and infection

Homozygous IL-15$^{-/-}$ mice on the C57BL/6 genetic background were bred at the University of Southern California and the Biological Resources Unit of the Cleveland Clinic as described (Zuo et al., 2005). C57BL/6 mice were purchased from the National Cancer Institute (Frederick, MD) at 5–6 weeks of age. Mice were infected between 6 and 8 weeks of age. Infections were initiated by intracerebral injection of 250 PFU of the J.2.2v-1 monoclonal Ab (mAb) neutralization derived JHMV variant as described (Fleming et al., 1986). For each experiment, mice were both sex- and age-matched, and groups of at least 3 individuals were analyzed per time point. No gender-dependent differences were observed. Infected mice were scored for clinical symptoms daily as described (Lin et al., 1997). All procedures were performed in compliance with protocols approved by the Institution Animal Care and Use Committees of the Keck School of Medicine and the Cleveland Clinic.

2.2. CNS cell preparations

CNS cells were isolated at various times post infection (p.i.) from brains or spinal cords (SC) of 3–6 mice per group, as described (Marten et al., 2000). Briefly, tissues were homogenized in ice cold Tenbrock homogenizers, adjusted to 30% Percoll (Pharmacia, Uppsala, Sweden) and layered onto a 1 ml 70% Percoll cushion. Following centrifugation at 800 ×g for 25 min at 4 °C, cells were recovered from the 30%/70% Percoll interface and resuspended in RPMI medium. Single cell suspensions were prepared from RBC-depleted spleens or CLN as described (Marten et al., 2000; Zuo et al., 2006).

2.3. Flow cytometry

Cell surface markers were examined as described (Ramakrishna et al., 2006; Zuo et al., 2005, 2006). Briefly, following blocking with anti-mouse CD16/CD32 (2.4G2, BD PharMingen, San Jose, CA), cells were stained with FITC-, PE- or CyChrome-conjugated mAb (for 3 color analysis), or FITC-, PE-, PerCP-, or APC-conjugated mAb (for 4 color analysis) using mAb specific for CD8 (53-6.7), CD44 (IM7), CD45 (30-F11), CD62L (MEL-14), CD69 (Hi.2F3) CD122 (TM-β1) (all from BD PharMingen), and CD127 (A7R34, eBioscience, San Diego, CA). Virus-specific CD8$^+$ T cells were detected with PE labeled D^b/S510 class I tetramer as described (Zuo et al., 2005). Stained cells were fixed with 2% paraformaldehyde and analyzed on a FACSCalibur flow cytometer (Becton Dickinson, Mountain View, CA). A minimum of 2.5×10^5 viable CNS cells were stained and $0.5–2\times10^5$ events per sample analyzed using CellQuestPro software (Becton Dickinson). 5×10^5 events were analyzed for low frequency signals in splenocytes. For intracellular granzyme B and Ki-67 detection, cells were first stained for surface Ag, then fixed and permeabilized with cytofix/cytoperm reagents (BD PharMingen), and stained with APC-labeled anti-granzyme B (GB12, isotype-control mouse IgG1) from Caltag Laboratories (Burlingame, CA), or FITC-labeled anti-Ki-67 (B56, isotype-control mouse IgG$_{1,\kappa}$ BD PharMingen). Intracellular IFN-γ was examined by stimulating 1×10^6 CNS cells in 200 μl of RPMI 1640 supplemented with 10% FCS, containing 1 μM S510 peptide (CSLWNGPHL) presented by EL-4 cells (Zuo et al., 2005; Zuo et al., 2006) and 1 μl/ml Golgistop (BD PharMingen) for 6 h. Cells were stained with anti-CD8 mAb, fixed, permeabilized with cytofix/cytoperm as described above, and IFN-γ was detected with anti-IFNγ mAb (XMG1.2) (BD PharMingen).

2.4. RNA isolation and PCR

One-half brains, spinal cords and spleens were homogenized in TRIzol (Invitrogen, Carlsbad, CA) using sterile Tenbroeck glass grinders. RNA was purified according to the manufacturer's protocol. DNA contamination was eliminated by treatment with DNAse using the DNA-free Kit (Applied Biosystems, Foster City, CA) according to the manufacturer's protocol in the presence of RNasin (Promega, Madison, WI). RNA integrity was confirmed by electrophoresis on 1.2% formaldehyde gels. Reverse transcription was performed on ≥2 μg RNA, primed with 1 μM random hexamers and oligo dTs (Promega, Madison, WI) using M-MLV reverse transcriptase (Invitrogen, Carlsbad, CA) for 1 h at 37 °C. Primers specific for HPRT were used to monitor RNA integrity. Real-time PCR was performed using a SYBR Green Master Mix (Applied Biosystems, Foster City, CA) and the following primers: IL-15: F, 5′-TGAGGCTGGCATTCATGTCTT-3′ and R, 5′-TCCAGTTGGCCTCTGTTTTAGG-3′; IL-7: F, 5′-AGTGCCACATTAAAGACAAAGAAGGT-3′ and R, 5′-ATTCGGGCAATTACTATCAGTTCCT-3′.

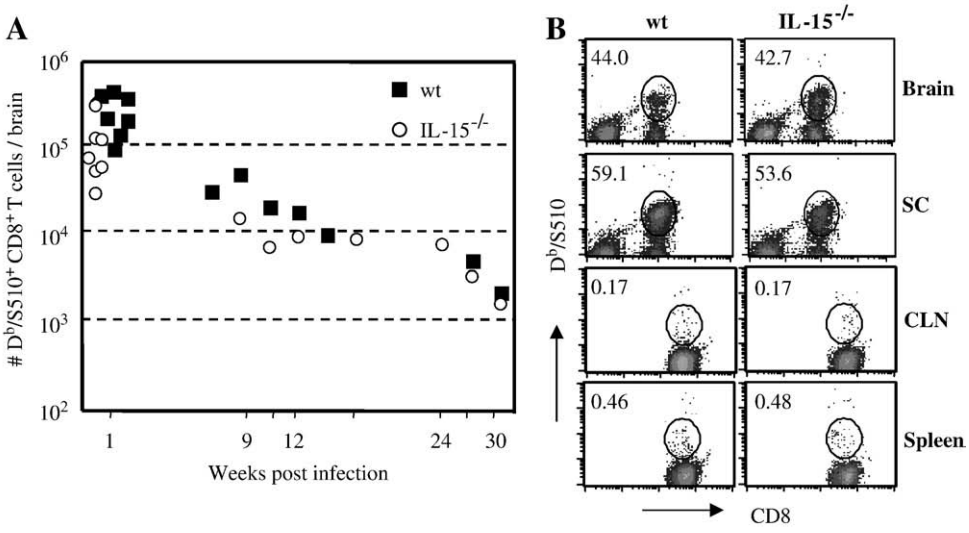

Fig. 1. Maintenance and distribution of virus-specific CD8$^+$ T cells during chronic CNS infection. A. Total numbers of D^b/S510 tetramer$^+$ CD8$^+$ T cells per brain at indicated time points p.i. Symbols represent numbers obtained from groups of pooled mice (n=3–5). Black squares depict wt and open circles IL-15$^{-/-}$ mice. B. Virus-specific memory CD8$^+$ T cells within brain, spinal cord (SC), CLN and spleen of wt and IL-15$^{-/-}$ mice (n=5/wt; n=4/IL-15$^{-/-}$) at 10 weeks p.i. Density plots are gated on either inflammatory CD45^{Hi} cells (brain and SC) or CD8$^+$ T cells (CLN and spleen). Numbers represent percentages of tetramer$^+$ cells (enclosed) within CD8$^+$ T cells. Data for spleens are from individual mice and depict the mean ± 0.06 for wt and ±0.17 (SD) for IL-15$^{-/-}$ mice. Results are representative of 3 time points during persistence.

Real-time PCR was performed using a MJ Research Opticon DNA engine, with Opticon Monitor software (Bio-Rad, Hercules, CA) under the following conditions: 95 °C for 15 min, 40 cycles of: denaturation at 94 °C for 20 s, annealing at 60 °C for 30 s and elongation at 72 °C for 20 s. All data presented are expressed as fold-induction based on the following formula: $(2^{(CT(GAPDH)-CT(TARGET\ GENE))})\times 1000$.

2.5. Statistical analysis

Means from two groups of mice were compared by two-tailed Student's *t* test and a statistically significant difference was indicated when P value <0.05.

3. Results

3.1. IL-15 independent maintenance of virus-specific CD8$^+$ T cells in the CNS

JHMV establishes a persistent CNS infection associated with low levels of persisting viral RNA, retention of T cells, and demyelination, but undetectable infectious virus (Bergmann et al., 2006; Marten et al., 2000). To determine whether IL-15 contributes to maintenance of CD8$^+$ T cells within the CNS, JHMV infected wt and IL-15$^{-/-}$ mice were examined between 1 and 30 weeks p.i. Total numbers of virus-specific D^b/S510 tetramer staining CD8$^+$ cells isolated from brains of IL-15$^{-/-}$ mice at one week p.i. were on average ~40% of those in wt mice (Fig. 1A). The lower absolute numbers of CNS recruited CD8$^+$ T cells reflect reduced naïve CD8$^+$ T cells in secondary lymphoid tissue of naïve IL-15$^{-/-}$ mice (Zuo et al., 2005). Nevertheless, the proportion of virus-specific cells within the CD8$^+$ T cell population was 37–50% in both groups, consistent with previous data (Zuo et al., 2006). Despite initially reduced total numbers of virus-specific CD8$^+$ T cells within the CNS, IL-15 deficiency did not accelerate their relative rate of contraction (Fig. 1A). Virus-specific CD8$^+$ T cells declined in both groups during persistence, with numbers in the CNS of IL-15$^{-/-}$ mice remaining at ~40% of wt levels out to 12 weeks p.i. (10 weeks: 7200 vs 21,100; 12 weeks: 9700 vs 18,300, respectively). Numbers of virus-specific CD8$^+$ T continued to slowly decline up to 30 weeks p.i., at which time cells from IL-15$^{-/-}$ mice approximated 70% of the wt numbers (Fig. 1A). In both groups the highest percentages of virus-specific specific CD8$^+$ T cells, constituting >50% of CD8$^+$ T cells, were detected in spinal cords (Fig. 1B), the primary site of viral RNA persistence (Marten et al., 2000). Similar to brains, the absolute numbers of virus-specific CD8$^+$ T cells in IL-15$^{-/-}$ spinal cords were lower, comprising within 30–50% the numbers recovered from wt mice. By contrast, virus-specific CD8$^+$ T cells in the spleen and CLN were <1% in both groups and no statistically significant differences were evident in spleens (Fig. 1B). Similar kinetics of infectious virus clearance in both mouse groups (Zuo et al., 2006) suggested that differential viral loads did

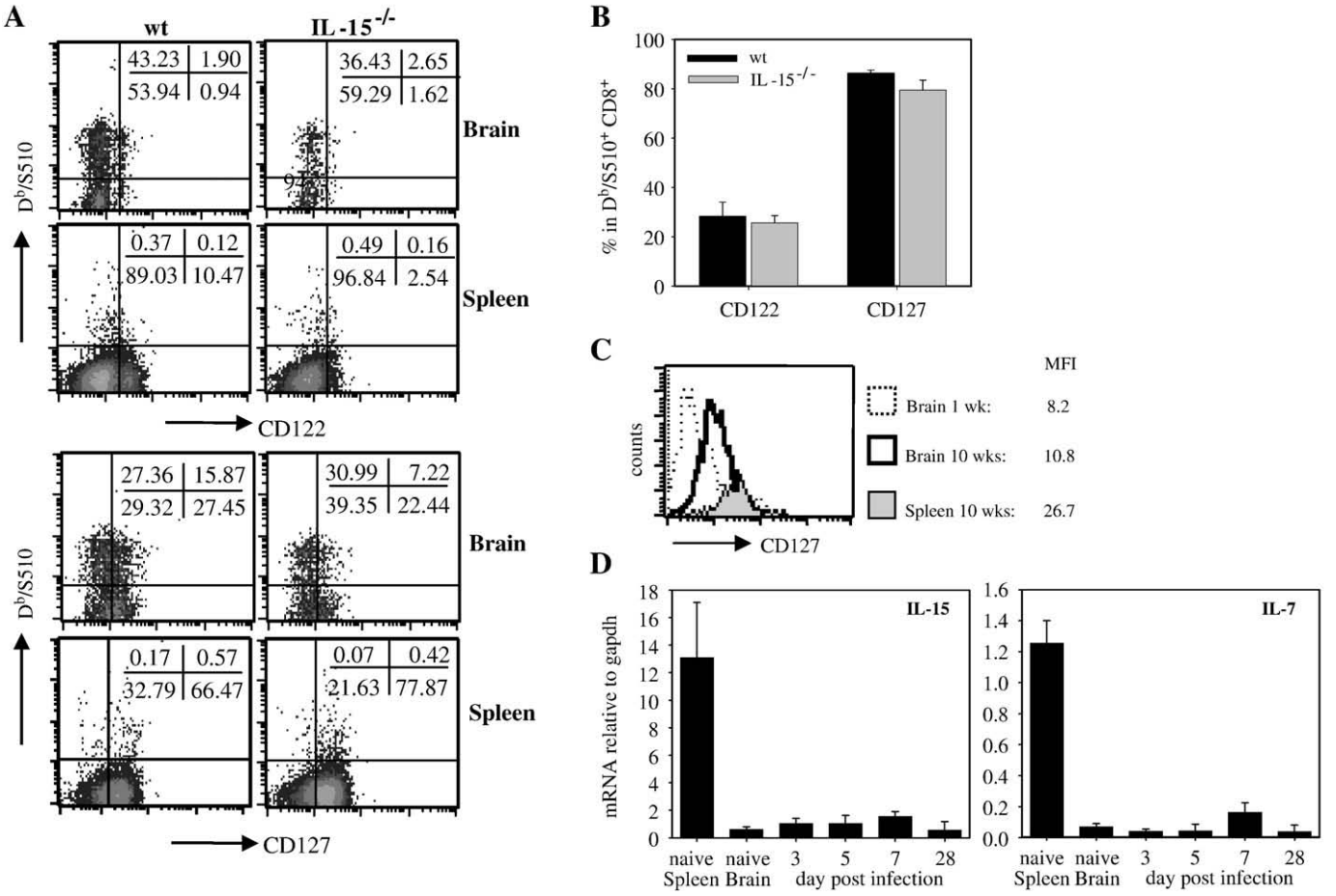

Fig. 2. Differential CD122 and CD127 expression on virus-specific CD8$^+$ T cells from brain and lymphoid tissue. A. Brain derived cells and splenocytes from wt and IL-15$^{-/-}$ mice were tested for CD122 or CD127 expression on D^b/S510 tetramer$^+$ CD8$^+$ T cells at 10 weeks p.i. Density plots from pooled samples (n=5/wt; n=4/IL-15$^{-/-}$) gated on CD8$^+$ T cells are shown. Numbers represent percentages in respective quadrants. B. Bar graphs represent mean percentages±SD of CD122$^+$ or CD127$^+$ cells within virus-specific tetramer$^+$ CD8$^+$ T cells from spleens of persistently infected wt mice (black, n=5) and IL-15$^{-/-}$ (open, n=4) mice at 10 weeks p.i. C. Relative CD127 expressions on tetramer$^+$ CD8$^+$ T cells in the brain of wt mice at 1 and 10 weeks p.i. are represented by dashed and thick lines, respectively. The shaded area depicts CD127 expression on wt splenic tetramer$^+$ CD8$^+$ T cells at 10 weeks p.i. for comparison. Respective mean fluorescence intensities (MFI) are shown. D. Spleens and brains from individual naïve or infected mice at the indicated times p.i. were analyzed for IL-15 or IL-7 mRNA expression by quantitative real time PCR (n=2–3 per group). RNA expression levels are normalized to the housekeeping gene GAPDH.

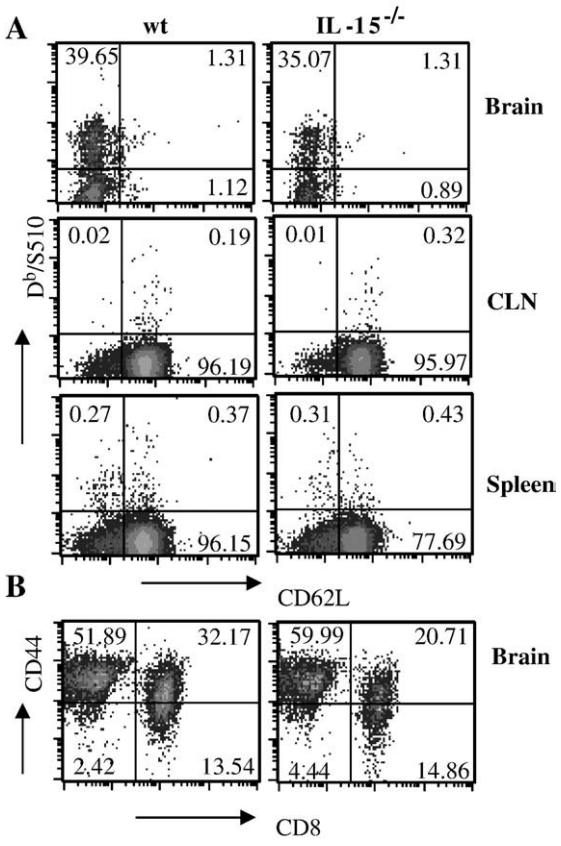

Fig. 3. CD62L and CD44 expression by virus-specific CD8$^+$ T cells persisting in the CNS. A. CD62L expression by virus-specific CD8$^+$ T cells in brain, CLN, and spleen of wt and IL-15$^{-/-}$ mice at 10 weeks p.i. (n=5/wt; n=4/IL-15$^{-/-}$). Density plots are gated on CD8$^+$ T cells. Numbers represent percentages within respective quadrants. B. CD44 expression on CNS CD8$^+$ T cells at 10 weeks p.i. Numbers represent CD44^{Hi} percentages within the CD8$^+$ T cell population. Representative of two experiments with similar results.

not affect CD8$^+$ T cell maintenance. Similar attrition of virus-specific CD8$^+$ T cells within the CNS of both groups suggested that their maintenance was independent of IL-15 as a survival factor.

3.2. IL-15 independent effector phenotype of CNS persisting CD8$^+$ T cells

Homeostatic proliferation and survival of memory CD8$^+$ T cells in lymphoid tissue are supported by IL-7 in addition to IL-15 (Surh et al., 2006). Persistent infections are associated with a sustained loss of CD127 expression on CD8$^+$ T cells (Lang et al., 2005; Paiardini et al., 2005) and an apparent reduced ability to generate memory CD8$^+$ T cells. Minimal levels of CD122 and CD127 are expressed by CD8$^+$ T cells entering the CNS during acute JHMV infection, despite prominent expression of CD122 in lymphoid tissue (Zuo et al., 2006). To test whether either receptor chain, particularly CD127, is re-expressed after CNS viral Ag diminishes, CD122 and CD127 expression was examined during chronic JHMV infection. In both wt and IL-15$^{-/-}$ mice <5% of virus-specific memory CD8$^+$ T cells within the CNS expressed CD122, consistent with IL-15 independent maintenance (Fig. 2A). Splenocytes from wt mice contained a larger frequency of CD122$^+$ tetramer$^-$ CD8$^+$ T cells compared to IL-15$^{-/-}$ mice (Fig. 2A), consistent with IL-15 dependent CD8$^+$ memory T cells in this compartment (Surh et al., 2006). However, the percentage of cells expressing CD122 in the tetramer$^+$ splenic CD8$^+$ T cells was similar at ~25–30% (Fig. 2A and B). CNS derived virus-specific CD8$^+$ T cells also remained predominantly CD127$^{-/Lo}$ (Fig. 2A), suggesting that IL-7 mediated signaling only partially compensates for IL-15 in maintaining CD8$^+$ T cells within the CNS. In stark contrast to the CNS, ~85% of virus-specific CD8$^+$ T cells in the spleen expressed CD127 in both wt and IL-15$^{-/-}$ mice (Fig. 2A and B), supporting differential maintenance mechanisms in these tissues. Although CD127 expression increased somewhat on the total CNS persisting CD8$^+$ T cell population compared to cells present during acute inflammation (Fig. 2C), expression levels did not reach those of splenic memory CD8$^+$ T cells (Fig. 2C). Low CD122 and CD127 expression on CNS derived CD8$^+$ T cells suggests that they maintain an effector phenotype. Analysis of IL-15 and IL-7 mRNA in the CNS of wt mice demonstrated low expression compared to spleens (Fig. 2D). Detection of basal IL-15 mRNA in the CNS of naïve mice is consistent with IL-15 expression in cerebellum and hippocampus (Gomez-Nicola et al., 2008). However, no significant increases of these cytokines in the CNS were observed during infection, supporting a minor role in driving CD8$^+$ T cell turnover within the CNS.

CNS derived CD8$^+$ T cells were essentially all CD62L$^{-/Lo}$ (Fig. 3A) consistent with an effector memory (T_{EM}) phenotype (Sallusto et al., 2000). Although the frequency of virus-specific CD8$^+$ T cells in CLN of

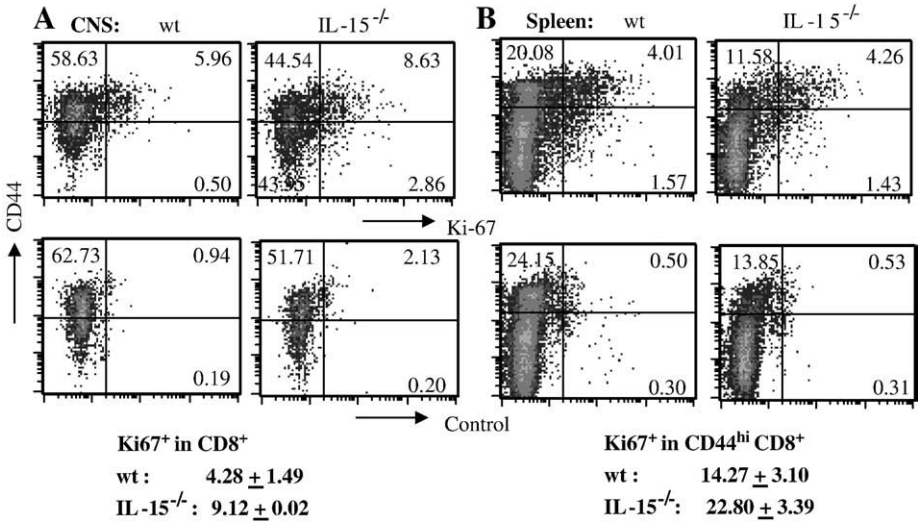

Fig. 4. Low proliferation of CNS derived CD8$^+$ T cells during chronic CNS infection. CNS derived CD8$^+$ T cells (A) and splenocytes (B) from infected wt (n=4) and IL-15$^{-/-}$ mice (n=4) were analyzed for intracellular expression of Ki-67 at 13 weeks p.i. Density plots gated on CD8$^+$ T cells show Ki-67 expression in combination with CD44 expression. Ki-67 expression by splenic CD44^{Hi} memory CD8$^+$ T cells is shown for comparison. Numbers indicate percentages in respective quadrants based on isotype control mAb. Numbers below representative plots depict mean percentages of Ki-67$^+$ cells in total CNS or splenic CD44^{Hi} CD8$^+$ T cells at 10 and 13 weeks p.i. after background staining was subtracted, ±SD.

both IL-15$^{-/-}$ and wt mice were extremely small, virtually all were CD62L^{Hi} (Fig. 3A). Cells in the spleen exhibited more heterogeneous CD62L expression, consistent with memory T cells established following control of peripheral infections (Wherry et al., 2003b). Confirming earlier data (Bergmann et al., 1999), CD69 expression was sustained by persisting CD8$^+$ T cells in the CNS of both wt and IL-15$^{-/-}$ mice to at least 12 weeks p.i. (data not shown). Although CD8$^+$ T cells express a CD44^{Hi} phenotype during acute inflammation (Bergmann et al., 1999), a significant proportion of CD8$^+$ T cells in both groups gradually down regulated CD44 by 10 weeks p.i. (Fig. 3B).

3.3. Limited homeostatic proliferation and gradual Bcl-2 up-regulation by CNS CD8$^+$ T cells

Attrition of persisting virus-specific CD8$^+$ T cells within the CNS, in conjunction with minimal CD122 and CD127 expression suggested low turnover rates. Consistent with this notion, only ~4% of total CNS persisting CD8$^+$ T cells in the CNS expressed Ki-67 in wt mice (Fig. 4A). Similar frequencies were detected within the tetramer$^+$ and tetramer$^-$ CD8$^+$ T cell populations (data not shown), negating selective turnover of virus-specific compared to CD8$^+$ T cells of unknown specificity. Although proliferation rates were increased almost 2-fold in the CNS of IL-15$^{-/-}$ mice, there was also no evidence for preferential proliferation of tetramer$^+$ CD8$^+$ T cells. Analysis of corresponding memory phenotype CD44^{Hi} CD8$^+$ T cells in the spleen demonstrated ~14% Ki-67$^+$ cells in wt mice and ~23% in IL-15$^{-/-}$ mice (Fig. 4B). The proportion of proliferating CD8$^+$ T cells in the CNS was thus reduced by 70% and 60% compared to splenic memory T cells in wt and IL-15$^{-/-}$ mice, respectively. An overall higher percentage of proliferating CD44^{Hi} cells in IL-15$^{-/-}$ compared to wt mice was also observed in naïve mice (data not shown), excluding persisting virus mediated proliferative renewal observed during latent herpes virus infection (Obar et al., 2004).

Long-lived memory CD8$^+$ T cells accumulate the anti-apoptotic molecule Bcl-2 (Grayson et al., 2000), while minimal Bcl-2 expression is associated with a high turnover rate during chronic Ag stimulation (Obar et al., 2004). Following virus clearance from the CNS of wt mice at 2 weeks p.i., CNS virus-specific CD8$^+$ T cells expressed low levels of Bcl-2 (Fig. 5). Bcl-2 expression gradually increased during persistence, accumulating to similar levels in both wt and IL-15$^{-/-}$ mice by 30 weeks p.i. (Fig. 5). Increasing Bcl-2 expression is consistent with a gradual decline in persisting viral RNA over time (Marten et al., 2000) and an increasingly resting CD8$^+$ T cell memory phenotype.

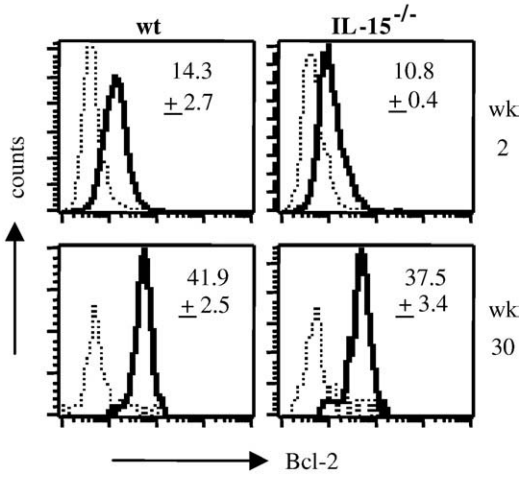

Fig. 5. Bcl-2 expression by virus-specific CD8$^+$ T cells during CNS persistence. Brain derived cells from wt and IL-15$^{-/-}$ mice ($n \geq 3$/group) were analyzed for Bcl-2 expression at 2 and 30 weeks following JHMV infection. Dashed lines and solid lines in histograms represent control and anti-Bcl-2 mAb reactivity on tetramer$^+$ CD8$^+$ T cells, respectively. Numbers represent mean MFI±SD of Bcl-2 expression from 2 (30 weeks) and three (2 weeks) separate experiments.

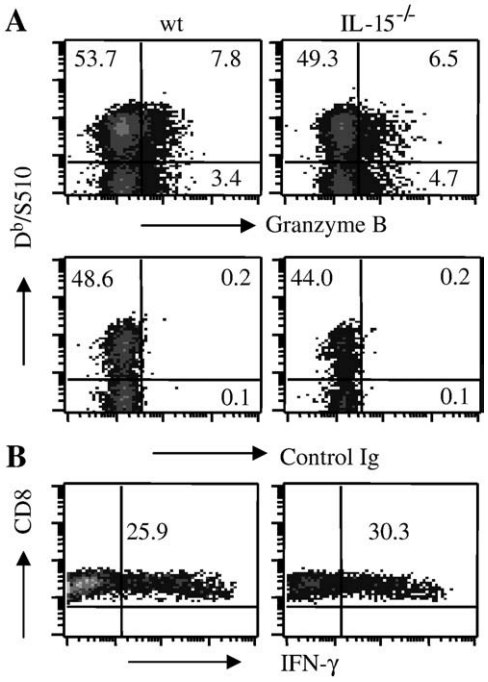

Fig. 6. Effector function of persisting virus-specific CD8$^+$ T cells in the CNS of wt and IL-15$^{-/-}$ mice. A. Pooled brain cells (n=5/wt; n=4/IL-15$^{-/-}$) were directly stained for intracellular granzyme B expression in tetramer$^+$ CD8$^+$ T cells at 10 weeks p.i. B. Brain cells at 10 weeks p.i. were analyzed for intracellular IFN-γ production following stimulation with S510 peptide presented by EL-4 cells (H-2D^b) for 6 h. Density plots are gated on CD8$^+$ T cells and numbers represent percentages within quadrants. Data in (A) and (B) are representative of two experiments.

3.4. IL-15 independent effector capacity of CNS residing CD8$^+$ T cells

An important property of CD8$^+$ T_{EM} cells or non-lymphoid tissue resident CD8$^+$ T cells is retention of effector functions, specifically constitutive expression of granzyme B (Masopust et al., 2001; Masopust et al., 2006). As IL-15 enhances granzyme B induction in memory CD8$^+$ T cells early upon Ag re-exposure (Yajima et al., 2005), CD8$^+$ T cells persisting in the CNS of both wt and IL-15$^{-/-}$ mice were thus compared for granzyme B expression and Ag-specific IFN-γ secretion. Virus-specific CD8$^+$ T cells from the CNS of both groups expressed low granzyme B levels (Fig. 6A), suggesting minimal cytotoxic capacity. These data are consistent with reduced granzyme B expression and impaired ex vivo cytolysis by virus-specific CD8$^+$ T cells derived from JHMV persistently infected BALB/c mice (Ramakrishna et al., 2004). However, only ~30% of virus-specific CD8$^+$ T cells in both wt and IL-15$^{-/-}$ mice secreted IFN-γ during persistence (Fig. 6B), compared to 60–70% during acute infection (Zuo et al., 2006). These data suggested that IL-15 does not contribute to the regulation of CD8$^+$ T cell effector function within the CNS during chronic infection.

4. Discussion

Factors governing a memory CD8$^+$ T cell pool are dependent on the history and environment of Ag exposure, as well as the tissue of residence (Bachmann et al., 2005; Ely et al., 2006; Masopust et al., 2006). During JHMV CNS infection virus-specific CD8$^+$ T cells are activated in CLN and preferentially accumulate in the CNS (Bergmann et al., 1999; Bergmann et al., 2006; Marten et al., 2000; Ramakrishna et al., 2006; Zuo et al., 2006). Although infectious virus is cleared, viral RNA and CD8$^+$ T cells persist in the CNS at low levels (Marten et al., 2000). The frequency of virus-specific CD8$^+$ T cells in lymphoid organs is barely detectable during chronic infection. This infection thus provides a model to examine T cell regulation at an effector site limited by the

constraints of the BBB, which is re-established following resolution of acute infection.

The paucity of CD122 expression by $CD8^+$ T cells entering the JHMV infected CNS (Zuo et al., 2006) suggested that T cells retained during persistence are IL-2 and IL-15 independent. IL-15 independent survival was supported by a persisting $CD122^{-/Lo}$ phenotype in the vast majority of CNS persisting $CD8^+$ T cells, as well as their IL-15 independent attrition. Similar percentages of virus-specific $CD8^+$ T cells in lymphoid tissue of wt and IL-$15^{-/-}$ mice during chronic infection was further consistent with their moderate CD122 expression in the spleen. This contrasted with high CD122 expression during the expansion phase (Zuo et al., 2006). Furthermore, while splenic JHMV specific $CD8^+$ T cells expressed CD127 during persistence, CNS persisting $CD8^+$ T cells expressed only low levels of CD127 in both wt and IL-$15^{-/-}$ mice. The persistence of JHMV specific $CD8^+$ T cells in the CNS contrasts the gradual loss of memory $CD8^+$ T cells from the respiratory tract attributed to IL-7R and IL-15R downregulation following influenza virus infection (Shen et al., 2008). Low level proliferation confirmed that IL-2, IL-7, and IL-15 do not play a major role in sustaining $CD8^+$ T cells within the CNS. Nevertheless, IL-7 likely contributes to sustain $CD127^+$ virus-specific T cells in lymphoid tissue in both wt and IL-$15^{-/-}$ mice, supporting a nonredundant role of IL-7 in homeostasis (Carrio et al., 2007). A possible stimulus contributing to $CD8^+$ T cell maintenance during persistent infection is persisting Ag (Obar et al., 2004; Wherry et al., 2003a). However, JHMV Ag is undetectable and viral RNA barely detectable in brains and spinal cords of wt mice by 6 weeks p.i. (Marten et al., 2000) providing little evidence for direct Ag mediated stimulation.

Consistent with characteristics of $CD8^+$ T_{EM} cells or memory $CD8^+$ T cells in non-lymphoid tissues, CNS $CD8^+$ T cells remained $CD62L^{-/Lo}$ and $CD69^+$ during persistent infection. However, they gradually down regulated CD44 expression, an adhesion molecule whose ligands include extracellular matrix proteoglycan and hyaluronate (Lesley et al., 1993). Virtually all inflammatory cells express high levels of CD44 during acute JHMV infection (Bergmann et al., 1999) consistent with CD44 mediated lymphocyte extravasation and entry into the CNS (Brennan et al., 1999). Down regulation of CD44 expression and gradual IL-15 independent Bcl-2 accumulation in CNS $CD8^+$ T cells implies local differentiation into long-lived memory $CD8^+$ T cells (Grayson et al., 2000). Consistent with a resting state, $CD8^+$ T cells expressing granzyme B were sparse throughout persistence. Prolonged CD69 expression by CNS T cells may thus reflect a retention rather than recent activation signal (Shiow et al., 2006). Indeed, there is no evidence for direct antiviral functions of $CD8^+$ T cells persisting in the CNS of JHMV persistently infected mice as demonstrated by the necessity for humoral immunity to control recrudescence, even in mice with established T cell memory (Ramakrishna et al., 2003; Ramakrishna et al., 2006). Furthermore, effector $CD8^+$ T cells persisting in the CNS, even when virus is cleared, are not necessarily associated with pathology (Hawke et al., 1998).

Ongoing recruitment and low level homeostatic turnover contributes to $CD8^+$ T cell maintenance in different anatomical compartments (Ely et al., 2006; Masopust et al., 2006). A major contribution of ongoing $CD8^+$ T cell recruitment into the CNS during JHMV persistence is unlikely due to limited BBB penetration by resting memory cells compared to other organs (Masopust et al., 2004). A decreased population of proliferating cells in CNS resident compared to splenic memory $CD8^+$ T cells, independent of Ag specificity, further supports minimal continual CNS recruitment. Finally, inhibition of chemokine signaling does not alter $CD8^+$ T cell frequencies during JHMV persistence (Stiles et al., 2006). These data favor the notion that CNS $CD8^+$ T cell populations are sustained as a result of low turnover.

IL-15 independent maintenance and function of $CD8^+$ T cells in the CNS contradict several previous reports demonstrating the importance of IL-15 for memory $CD8^+$ T cell homeostasis, survival and even cytotoxicity (Becker et al., 2002; Obar et al., 2004; Saito et al., 2006; Yajima et al., 2005; Yajima et al., 2006). This dichotomy is most evident in the association between enrichment and persistence of virus specific $CD8^+$ T cells with elevated IL-15 levels in the CNS of simian immunodeficiency virus infected monkeys (Marcondes et al., 2007). Differences may reside in virus tropism, type I IFN mediated upregulation of IL-15, as well as persistent virus load. Our results support basal expression of IL-15 in the CNS (Gomez-Nicola et al., 2008), yet levels are significantly reduced relative to lymphoid tissue. The absence of significantly enhanced IL-15 expression during infection is consistent with low levels of type I IFN induction by coronaviruses (Ireland et al., 2008; Roth-Cross et al., 2008). Although we cannot rule out that loss of in vivo $CD8^+$ T cell function during persistence may be attributed to the sparse IL-15 environment, IFN-γ secretion was effectively initiated after short term stimulation in vitro without supplementing IL-15. The recent findings that IL-15 enhances activation of astrocytes and microglia (Gomez-Nicola et al., 2008), furthermore caution that IL-15 supplementation in vivo to enhance CD8 T cell immunity in the CNS may contribute to gliosis and pathology.

In summary, these data indicate that $CD8^+$ T cells persisting within the JHMV infected CNS are IL-15 independent and derived from effector $CD8^+$ T cells recruited into the CNS during peak inflammation. The CNS persisting population slowly adopts a mixed phenotype with characteristics of both T_{EM} and T_{CM}. High CD127 expression on memory $CD8^+$ T cells in lymphoid tissue, but not on CNS persisting $CD8^+$ T cells further suggests a minor role of IL-7 in CNS T cell maintenance. The CNS thus has a unique capacity for long term maintenance of memory CD8 T cells largely independent of IL-15 and IL-7.

Conflict of interest

The authors have no financial conflict of interest.

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