A TH1 immune response efficiently clears rabies virus from the CNS in the absence of inflammation

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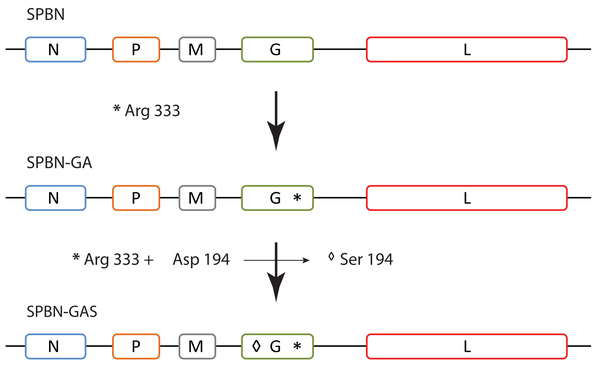
1. Introduction

Little is known about CD4+ T cell entry into the central nervous system (CNS) in the absence of inflammation. Attenuated rabies viruses (RABV) are unique tools to study this process, as they spread from the site of inoculation to the CNS trans-axonally, without compromising the blood-brain barrier. Previous studies with live-attenuated RABV showed that CD4+ T cell entry into the CNS is associated with an increase in IFN-g mRNA in the brain and the production of IgG2a antibodies in the periphery, indicating a bias towards TH1 immunity. To further investigate the nature of the CD4+ T cells involved in the CNS response to RABV, we performed a temporal comparison of development of antiviral immunity in C57BL/6 and T-Bet knock-out (T-Bet$^{-/-}$) mice. In both mouse strains, we observed that virus spread through the CNS is associated with elevated IFN-g mRNA, but that immune cell infiltration and antibody production in CNS tissues differs significantly. Immune cell infiltration into the CNS is significantly lower in T-Bet$^{-/-}$ mice at day 8 post-infection. Moreover, despite the fact that immunized T-Bet$^{-/-}$ mice produce a substantial amount of neutralizing antibody in the periphery and are fully protected against i.m. challenge with pathogenic RABV, they are unable to survive an i.c. challenge with the same virus. Together, these findings suggest that TH1 cells play a critical role in cell infiltration into the CNS and the clearance of RABV despite the absence of inflammatory pathology.

Funding: supported by grants from the NIH (AI093369 and AI083046)

2. Experimental design

Rabies virus strain

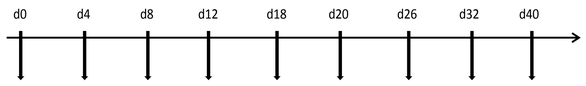


SPBN-GAS is a genetically engineered attenuated rabies virus. *Milosz Faber et al. , Journal of Virology, Nov. 2005*

Time course experiment

C57 BL/6 and T-bet $^{-/-}$ (10 mice per group) were infected intranasally with 10^5 FFU of SPBN-GAS virus

Samples were collected at the following time points after infection



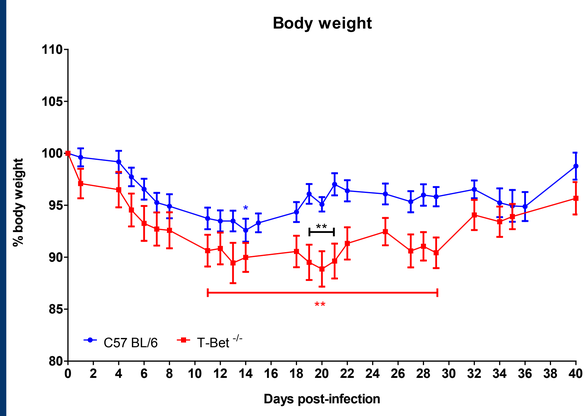
➔ Efficacy of infection was assessed by mice weight loss

➔ Antibody production in the perphery was assessed by ELISA

➔ Immune cell accumulation into the cortex was estimated by the levels of cell marker mRNA determined by quantitative PCR

3.1 Virus spread

A



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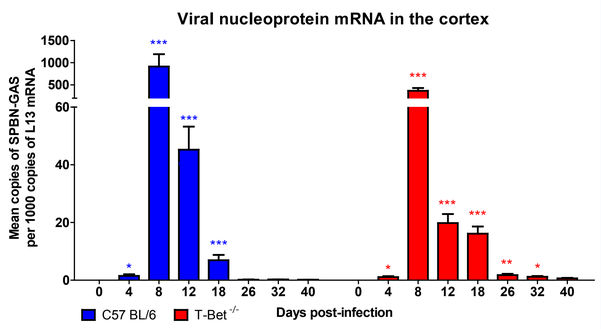


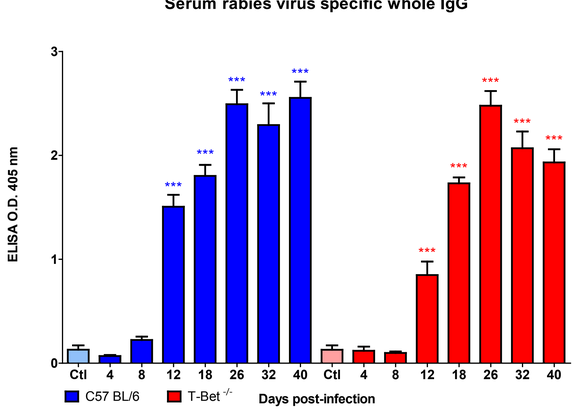
(A) C57 BL/6: transient weight loss (~7%), significant at day 14 post-infection. T-Bet $^{-/-}$: weight loss between day 11 and day 29 (~12%). C57 BL/6 mice regain their weight faster than T-Bet $^{-/-}$ mice.

(B) Peak of virus nucleoprotein mRNA at day 8 after infection for both strains of mice. Copies of virus mRNA persist longer in the cortex of T-Bet $^{-/-}$ mice.

Statistics: the result for each time point was compared to the strain-matched controls with the non-parametric Kruskal-Wallis test, followed by Dunn's mulitple comparison test. * p-value ≤ 0.05; ** p-value ≤ 0.01; *** p-value ≤ 0.005

3.2 Peripheral immune response

A



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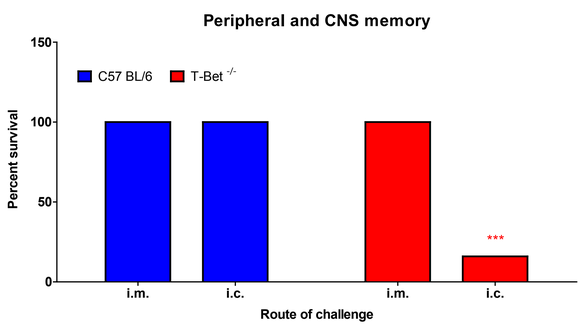
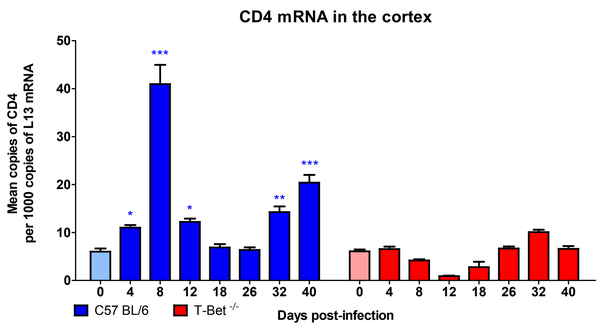


(A) Serum dilution 1/40. Both strains of mice have significant levels of serum IgGs specific for SPBN-GAS from 12 days after infection. Statistics: test sera were compared to strain-matched controls with the parametric One Way ANOVA test, followed by a Bonferroni's multiple comparison test. *** p-value ≤ 0.005

(B) Mice were immunized in the leg with SPBN-GASGAS virus (apathogenic) and challenged either i.m. or i.c., 14 days after immunization, with DOG4 virus (pathogenic). C57 BL/6 mice are protected from the challenge independantly of its route of administration. T-Bet $^{-/-}$ are protected from a peripheral but not a CNS challenge. Statistics: Fisher's exact test. *** p-value ≤ 0.005

3.3 T cell accumulation in the cortex

A



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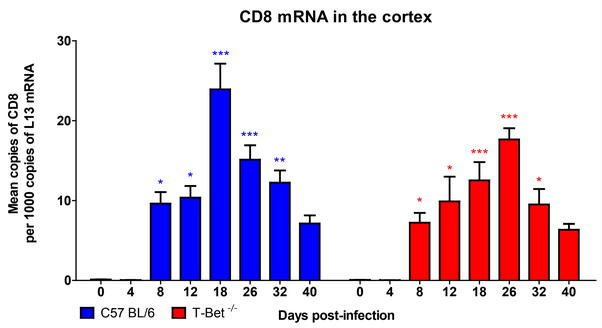

(A) C57 BL/6: CD4 T cells accumulate from day 4 to 12 after infection with a peak at day 8 , followed by a second wave of cell entry and/or cell division 32 days after infection. T-Bet $^{-/-}$: no CD4 T cell accumulation in the cortex.

(B) Strong and sustained accumulation of CD8 T cells from 8 days up to 40 days after infection in both mouse strains, with a peak at day 18 post-infection for C57 BL/6 mice, and at day 26 post-infection for T-Bet $^{-/-}$ mice.

Statistics: mRNA levels were compared to their strain-matched controls with the non-parametric Kruskal-Wallis test, followed by Dunn's mulitple comparison test. * p-value ≤ 0.05; ** p-value ≤ 0.01; *** p-value ≤ 0.005

3.4 B cell accumulation and antibody production in the cortex

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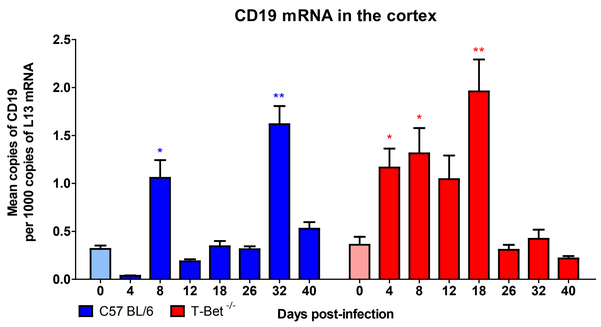

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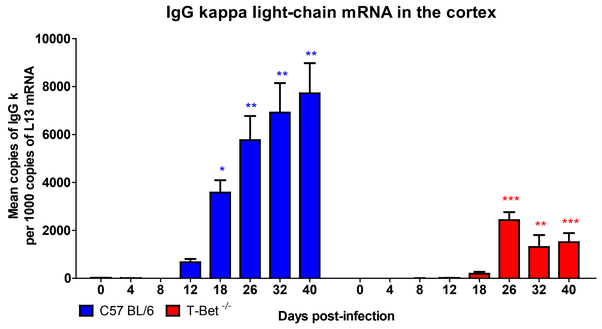

(A) C57 BL/6: B cells initially appear at day 8, followed by a sacond wave of cell entry and/or cell division at days 32 post-infection. T-Bet $^{-/-}$: more extensive accumulation of B cells in the cortex between day 4 and 18 after infection.

(B) C57 BL/6: antibody production starts at day 12 post-infection and progressively increase until day 40 post-infection. T-Bet $^{-/-}$: late and lower antibody production in the cortex appearing around 26 days after the infection.

Statistics: mRNA levels were compared to their strain-matched controls with the non-parametric Kruskal-Wallis test, followed by Dunn's mulitple comparison test. * p-value ≤ 0.05; ** p-value ≤ 0.01; *** p-value ≤ 0.005

4. Conclusions

	C57 BL/6	T-Bet $^{-/-}$
Early virus spread into the CNS	+++	++
Late virus spread in the CNS	+	+++
Peripheral antibody response	+++	+++
CD4 T cell accumulation	+++	-
CD8 T cell accumulation	+++	+++
B cell accumulation	+	+++
Antibody production in the CNS	+++	+
Peripheral memory	+++	+++
CNS memory	+++	-