7 TESLA MR SPECTROSCOPY REVEALS THAT CD8 T LYMPHOCYTE DEPLETION ALONE HAS NO EFFECT ON BRAIN METABOLITE CONCENTRATIONS CONFIRMING THE ACCELERATED RHESUS MACAQUE MODEL OF NEUROAIDS

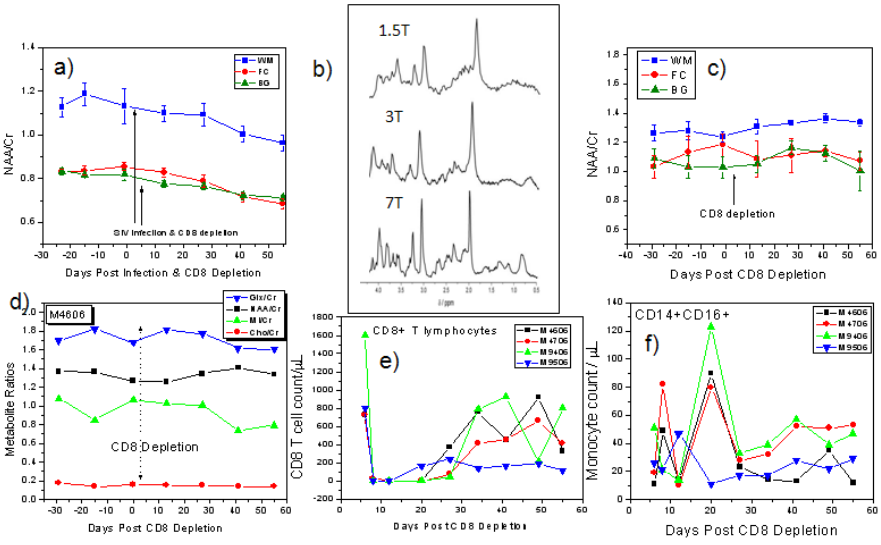
E-M. Ratai[1,2], S. Pilkenton[1,2], J. Bombardier[1], C-G. Joo[1,2], K. W. Turk[1], M. R. Lentz[1,2], J. He[1,2], L. Annamalai[2,3], S. O' Neil[2,3], S. V. Westmoreland[2,3], T. H. Burdo[4], J. H. Campbell[4], C. Soulas[4], P. Autissier[4], W-K. Kim[5], K. Williams[4], and R. G. Gonzalez[1,2]

[1]Radiology, Massachusetts General Hospital - A.A. Martinos Center for Biomedical Imaging, Charlestown, MA, United States, [2]Harvard Medical School, Boston, MA, United States, [3]New England Regional Primate Research Center, Southborough, MA, United States, [4]Biology Department, Boston College, Boston, MA, United States, [5]Eastern Virginia Medical School, Norfolk, VA, United States

Introduction: The applications of *in vivo* CD8 monoclonal antibodies are manifold and include harnessing and amplifying the natural tolerance mechanism to control responses of foreign tissue after organ transplantation [1]. In addition, a transient depletion of T lymphocytes results in reduction of tumor cell metastases [2]. In animal models, CD8 depleting antibodies have been used to understand the immune mechanisms to improve vaccines for controlling the measles virus [3] and to accelerate disease progression in other infectious diseases such as HIV [4].

Despite the success of antiretroviral therapies, the cognitive complications of HIV infection (neuroAIDS) continue to be an important problem. The best animal model, the SIV infected macaque, has been highly informative, but it is limited by the fact that SIV encephalitis (SIVE) occurs in less than a third of infected animals, and requires a lengthy time course of two or more years. The SIV-infected, CD8+ T lymphocyte depleted macaque model method results in a reliable accelerated model of neuroAIDS: >90% of animals remain persistently depleted, and of these animals >95% demonstrate histopathological signs of SIVE within 8-10 weeks of infection similar to HIV encephalitis including the accumulation of viral-laden perivascular macrophages and multinucleated giant cells, astrogliosis, microgliosis, and neuronal injury. Moreover, MRS changes are very similar. Thus, this model is well suited for effective treatment testing. In addition, using this accelerated model, we have demonstrated our ability to manipulate the severity of this damage with antiretroviral therapy [5]. However, to validate this animal model it is of vital importance to prove that the virus is in fact damaging the brain, and that CD8 depletion alone has no significant effect on brain metabolite concentrations.

Methods: Eight rhesus macaques (*macaca mulatta*) were used in this study. Four rhesus macaques were treated with 3 doses of an anti-CD8 antibody (cM-T807) administered s.c. (10 mg/kg) and 2 and 6 days later i.v. (5 mg/kg) to deplete CD8 T lymphocytes. Four animals were inoculated with the simian immunodeficiency virus (SIVmac251) and CD8 depleted at 6, 8 and 12 days post infection (d.p.i.). Flow cytometry was used to monitor CD8+ T lymphocyte depletion and to determine other lymphocyte concentrations such as CD4 and monocyte populations (CD14 and CD16). Animals were examined with MRI and MRS 2-3 times before and biweekly after CD8 depletion or SIV infection until 8 weeks post depletion. In *vivo* studies were performed on a 3.0 T and 7.0 T Siemens MRI scanners using a CP extremity coil and an 18 cm TEM coil, respectively. Single voxel 1H MR spectra were acquired from the subcortical white matter (WM), frontal cortex at the midline (FC), and the basal ganglia (BG) using a point resolved spectroscopy (PRESS) sequence with TE/TR = 30/2500ms. Metabolite concentrations N-Acetyl-aspartate (NAA), choline (Cho), myo-Inositol (MI), creatine (Cr) and glutamine/glutamate (Glx) were quantified using the LCModel software package as ratios over Cr and using the unsuppressed water peak as reference. Repeated measures analysis of variance and Holm's t-tests were performed using JMP 7.0 (SAS, Cary, NC). **Results:** The four SIV infected rhesus macaques were scanned at a 3T MRI scanner. SIV infection and CD8 depletion resulted in a rapid decline in NAA/Cr levels a marker of neuronal health in all three brain regions (WM -17% p<0.0001; FC -20% p<0.0001; BG -13% p=0.004) indicating neuronal injury (Figure 1a). Cho/Cr (WM p=0.014, FC p<0.0001, and BG p=0.008) and MI/Cr (WM p=0.003, FC p<0.0001, and BG p=0.011), proposed markers of inflammation displayed an initial increase at 2 weeks post infection and then decreased back to baseline values or below. Cr linearly increased over time in WM (p=0.013 +10%) and Cho increased once more 8 weeks p.i.(WM p=0.02 +10%).



To improve the sensitivity in this study, we performed the control animal study on a 7T scanner to make sure we would detect changes due to CD8 depletion, if indeed present. Figure 1b shows MRS data obtained from macaque brains at three different field strengths using similar parameters. As anticipated, higher field strengths resulted in better signal to noise ratio, higher spectral resolution, and therefore improved quantification precision. Figure1c shows the mean NAA/Cr ratios of the four CD8 depleted animals, in the WM, FC and BG as a function of time post CD8 depletion. We found no significant changes in any of the brain regions over time and conclude that CD8 depletion alone has no significant effect on neuronal integrity in the brain. In addition, we evaluated the changes of Cho/Cr, MI/Cr and Glx/Cr before and after CD8+ T lymphocyte depletion, and found no statistically significant changes due to CD8+ T lymphocyte depletion. Figure 1d shows these changes in one representative animal over time. None of these metabolite ratios showed any change due to the anti-CD8 treatment. In addition, the metabolite concentration in institutional units also did not change with CD8 depletion. All four animals were persistently CD8 depleted (>21 days) (Figure 1e). Flow cytometric analysis demonstrated an increase in the percentage monocytes (p=0.009) and an expansion of CD14+CD16+ monocyte population 21 days post depletion (p=0.048) (Figure 1f).

Conclusions: MR spectroscopy at 7 Tesla revealed that metabolite concentrations ratios did not change with anti-CD8 treatment even an appropriate immune response was observed extra cranially where an increase in monocyte population was observed. These findings confirm the validity of our accelerated rhesus macaque model of neuroAIDS and also prove that despite manipulating the immune system brain metabolism is conserved.

References: [1] Waldmann et al, Annu. Rev. Immunol. 1998;16:619, [2] Rasku et al, J Transl Med. 2008;6:12, [3] Permar et al, JID 2004;190:998, [4] Schmitz et al, Science 1999;283(5403):857, [5] Williams at al. J Clin Invest. 2005;115:2534

Acknowledgements: E. Moeller, S. Luboyeski, D Raikowsky, M Duggan, and J. Morris, for animal care and NIH grants R21NS059331, R01NS050041, R01NS040237, P41RR014075, and MIND Institute