Contrasting roles for CD4 and CD8 T cells in a murine model of T1 black hole formation

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Objective: To determine the role of CD8+ and CD4+ T-cells in the formation of T1 hypointense lesions (T1 black hole, T1BH) in a murine model of MS.
Background: TMEV infection in mice represents an accepted model of multiple sclerosis.[1] In C57B6/J mice, infection with Theiler's Murine Encephalomyelitis Virus (TMEV) results in the formation of T1 hypointense lesions in the cerebrum.[2] In this model, CD 8 T cells are the main contributors in the pathogenesis of T1 hypointense lesions. The goal of this study was to establish whether the CD8 T-cells are classic viral epitope specific cytotoxic lymphocytes or other CD8 positive immune cells, and to investigate the potential role of CD 4 T cells in the process of T1BH formation. CD 4 T cells are considered significant mediators in the pathogenesis of multiple sclerosis and its key animal model, EAE (experimental allergic encephalomyelitis). While subsets of CD 4 T cells are common targets in therapeutic efforts directed at MS, recent pathology reports also indicate a role for CD 8 T cells, which appear to be the most numerous lymphocyte population in MS lesions, regardless of stage of lesion formation. A subset of these cells were observed in close proximity to damaged neurons in axons.[3] Animal models are of great importance in clarifying the role and significance of CD 8 vs. CD 4 lymphocytes in neuroinflammatory diseases. In addition, since T1BH are thought to represent axonal/neuronal damage in MS[4], and correlate better with a disabling disease course compared to T2 hyperintense lesion load, these investigations may pave the way to a clearer understanding of the substrate of disability in MS.
Design and Methods: The experiments were approved by the Institutional Animal Care and Use Committee. In the experiments determining whether epitope specific VP2 cells are contributing to the process, we used epitope depletion: it is known that over 60% of brain infiltrating CD8 T cells in TMEV infected C57B6/J mice recognize the VP2 epitope, and that intravenous administration of this peptide one day prior to infection depletes this cell population.[5] In this study, 8 TMEV infected mice underwent VP2 depletion, and 8 TMEV infected mice received E7 peptide injection as negative controls. The E7 peptide is derived from the human papilloma virus, which has no relevance in TMEV infection. To determine the role of CD8 and CD4 T cells in general, we utilized mice that lack an adaptive immune system, and as such, they lack CD 4 and CD 8 T cells. These Rag-1 deficient mice otherwise have the same C57B6/J genetic background. We harvested CD8 and CD 4 T cells from spleens of C57B6/J mice, and transferred these into Rag-1 deficient recipient mice. A total of 8 mice received CD8 transfer, another 8 received CD4 transfer; while 8 control mice received sham transfer of PBS solution that contained no cells. In each case, the formation of T1BH was monitored by volumetric MRI: a volume acquisition T1 weighted spin echo sequence was used, with 200µm isometric resolution. For volumetric analysis, the 3D ROI tool was used in Analyze 9.1 (Mayo Clinic, Biomedical Imaging Resource). Since the analysis is semi-automated, 2 investigators analyzed each datasets at least twice, with over 95% agreement between their measurements. The outcome analyzed in our studies was the total volume of T1BH per animal.
Results: . The cell transfer experiments revealed that CD8 T cell recipient mice developed an average T1BH volume over 3 fold higher compared to sham transfer controls (p=0.0002) (Figure 1). The CD 4 transfer experiments demonstrated a very surprising finding: CD4 T cells actively prevented T1BH formation in our model: an almost 4-fold reduction (p=0.0006) in T1BH volume compared to sham treated irradiated infected controls. Depleting the CD8 T cells that recognize the VP2 immunodominant peptide (Figure 2) resulted in a 2-fold reduction of T1BH formation compared to sham treatment (p=0.017). While this is a very significant reduction, our findings also suggest that not only VP2 epitope specific classic CD8 T cells, but other immune cells/mechanisms also play a minor role in the formation of T1BH.
Conclusions/Relevance: The above results demonstrate that (1) CD8 T cells play the predominant role in the pathogenesis of T1BH formation, with the leading contribution by epitope specific cytotoxic CD8 T cells. Since a small degree of T1BH formation is observed even in control animals (infected Rag-1 mice receiving sham transfer) it is likely that either direct cytopathic effects or cells of the innate immune system also contribute to this process, while (2) CD4 cells appear to play a preventive role in this process. Considering that T1BH formation is thought to represent axonal and/or neuronal damage in MS, cytotoxic CD8 T cells targeting axons and/or neurons may be the mediators of this finding in neuroinflammatory diseases. The surprising preventive role of CD4 T cells may be explained by recent observations that Th17 cells (a subset of CD4 T cells) prolong neuronal survival in the TMEV model in vitro.[6] Our novel findings demonstrate a contrasting role for CD4 vs. CD8 cells in T1BH formation in this murine model.
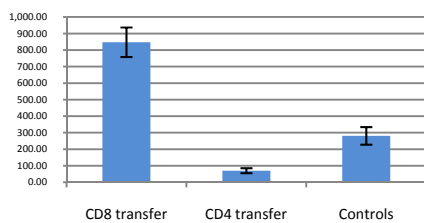
Figure 1. MRI T1BH volumetry results in adoptive transfer experiments using RAG 1 -/- recipient mice.
The bar chart shows the mean T1BH volumes in CD8 and CD4 adoptive transfer experiments. Controls represent sham transfer (PBS injection) following irradiation and infection. CD8 T cell transfer resulted in 3-fold increase of T1BH formation, while CD4 transfer resulted in a 2-fold decrease. Y axis: volume units in $0.01mm^3$. Error bars represent SD.

Figure 2. MRI T1BH volumetry results following peptide depletion.
VP2 is the viral epitope recognized by over 75% of all CNS infiltrating CD8 T cells in the context of TMEV infection of C57BL6/J mice. E7 is an irrelevant viral petide. Note the significant reduction in T1BH formation by peptide-induced depletion (injection of VP2 peptide 1 day prior to infection). Y axis: volume units in $0.01mm^3$. Error bars represent SD.

References

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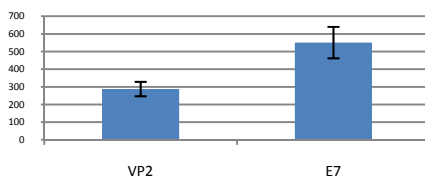6. Hou W, J Exp Med 2009;206(2):313-328.