

Clonally expanded CD8 T cells patrol Alzheimer's cerebrospinal fluid

David Gate^{1,2}, Naresha Saligrama³, Olivia Leventhal¹, Andrew C. Yang^{4,5}, Michael S. Unger^{6,7}, Jinte Middeldorp^{1,2,8}, Kelly Chen¹, Benoit Lehallier^{1,2}, Divya Channappa¹, Mark B. De Los Santos¹, Alisha McBride^{1,2}, John Pluvinage^{1,9,10}, Fanny Elahi¹¹, Grace Kyin-Ye Tam^{1,12}, Yongha Kim^{1,12}, Michael Greicius^{1,12}, Anthony D. Wagner^{13,14}, Ludwig Aigner^{6,7}, Douglas R. Galasko¹⁵, Mark M. Davis^{3,16,17}, *Tony Wyss-Coray^{1,2,5,14,18}

¹Department of Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford, California, USA.

²Veterans Administration Palo Alto Healthcare System, Palo Alto, CA, USA.

³Department of Microbiology and Immunology, School of Medicine, Stanford University, Stanford, California, USA.

⁴Department of Bioengineering, Stanford University, Stanford, California, USA.

⁵Chemistry, Engineering, and Medicine for Human Health (ChEM-H), Stanford University, Stanford, California, USA.

⁶Institute of Molecular Regenerative Medicine, Paracelsus Medical University, Salzburg, Austria.

⁷Spinal Cord Injury and Tissue Regeneration Center Salzburg (SCI-TReCS), Paracelsus Medical University, Salzburg, Austria.

⁸Department of Translational Neuroscience, University Medical Center Utrecht Brain Center, Utrecht University, Utrecht, The Netherlands

⁹Medical Scientist Training Program, Stanford University School of Medicine, Stanford, CA, USA.

¹⁰Stem Cell Biology and Regenerative Medicine Graduate Program, Stanford University School of Medicine, Stanford, California, USA.

¹¹Department of Neurology, Memory and Aging Center, University of California at San Francisco (UCSF), San Francisco, California, USA.

¹²Functional Imaging in Neuropsychiatric Disorders (FIND) Lab, Department of Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford, California, USA.

¹³Department of Psychology, Stanford University, Stanford, California, USA.

¹⁴Wu Tsai Neurosciences Institute, Stanford University, Stanford, CA, USA. Stanford Neurosciences Institute, Stanford University, Stanford, California, USA.

¹⁵Department of Neurosciences, University of California, San Diego, La Jolla, California, USA.

¹⁶Institute for Immunity, Transplantation and Infection, Stanford University School of Medicine, Stanford, California, USA.

¹⁷Howard Hughes Medical Institute, Stanford University School of Medicine, Stanford, California, USA.

¹⁸Paul F. Glenn Center for the Biology of Aging, Stanford University School of Medicine, Stanford, California, USA.

*Corresponding author: twc@stanford.edu

Supplementary Table 1. AD T cell literature.

A list of publications focused on AD adaptive immunity with key findings, methodologies and sample sizes for each study.

Supplementary Table 2. Cohort characteristics.

Sample size, sex characteristics, average age, ethnicity, education and cognitive score data are provided for study cohorts.

Supplementary Table 3. Markers used for immune profiling by mass cytometry.

Markers used in mass cytometry experiments included 21 cell surface antibodies and DNA interchelators.

Supplementary Table 4. CITRUS model features.

P-values a derived from comparing MCI/AD (n=23) vs. healthy (n=23) subjects; unpaired two-sided *t-test*.

Supplementary Table 5. Significance of diagnosis on classical immune variables reveals alterations in CD8⁺ T cells.

Dependent variables are listed, with corresponding p-values, marginal means, standard error and 95% confidence interval values for each group (n=57 healthy, n= 23 MCI/AD; unpaired two-sided *t-test* with Bonferroni correction).

Supplementary Table 6. Characteristics of subjects used for post-mortem immunohistochemistry analysis.

Age, sex, post-mortem interval and pathology notes for each subject used for histology in cohort 3.

Supplementary Table 7. TCR sequences from plate-seq experiments.

T cell receptor sequences of each subject from plate-seq.

Supplementary Table 8. Differentially expressed genes of several of the most highly expanded clones that have been previously associated with AD.

Published studies related to differentially expressed genes of top AD T cell clones.

Supplementary Table 9. GLIPH results from clustering AD CSF plate-seq TCRs.

GLIPH algorithm results from plate-seq experiments.

Supplementary Table 10. List of 80 MHC-I peptides included in the pool used in antigen presentation experiments.

Amino acid lengths, species and antigens of the 80 MHC-I peptides used in antigen screening experiments.

Supplementary Table 11. List of candidate peptides, sequence, antigen name and their HLA restriction for 15 candidate peptides used in antigen presentation experiments.

Amino acid lengths, species, antigens and HLA specificity of peptides used in TCR stimulation experiments.

Supplementary Table 12. Group characteristics for all study subjects.

Group sizes, average age, sex, ethnicity, education, cognitive scores, APOE genotypes and biomarker levels of all study subjects.