

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Chen Y, Bord E, Tompkins T, et al. Asymptomatic reactivation of JC virus in patients treated with natalizumab. *N Engl J Med* 2009;361:1067-74.

To detect JCV VP1-specific T cells, IFN- γ ELISPOT assay was performed, using a JCV VP1 protein library consisting of ninety-seven 15aa peptides overlapping by 11aa spanning the entire VP1 protein, divided into four sequential pools, as follows: Pool A: p1-p93 (n=24); Pool B: p97-p157 (n=24); Pool C: p161-p253 (n=24); Pool D p257-p341 (n=25). This assay allowed us to define the presence of JCV-peptide-reactive T cells regardless of HLA alleles of the study subjects. Briefly, 7×10^6 freshly isolated PBMC were incubated in RPMI 1640/12% fetal calf serum (FCS) medium with each overlapping peptide pool A-D at 2 μ g/ml final concentrations for 72h, followed by supplementation with 25U IL-2 every other day for 10-14 days. All cultures were washed with 2% FCS PBS and re-suspended in medium without IL-2 for 16-24 hours before being assayed. On the day of assay, the exact number of PBMC in cultures was determined using an automated cell counter (Guava Technologies, Hayward, CA).

The ELISPOT assay was performed as described elsewhere* according to the manufacturer's protocol (Mabtech). Briefly, 96-well Multiscreen-IP plates (Millipore) were coated with 5 μ g/ml mouse anti-human IFN- γ mAb overnight at 4°C. 10^5 cells/well from each peptide pool cultures were incubated with PHA-M, media alone, or re-stimulated with the same peptide pools or with media alone in triplicate overnight at 37°C, at a final concentration for each peptide of 2 μ g/ml. Spot forming units (SFU) were quantified with an automated ELISPOT plate reader (Hitech Instruments) using Image-Pro Plus image-processing software (version 4.1) (Media Cybernetics, Des Moines, IA) and quality-controlled by a dedicated analyst. Frequencies of JCV VP1 specific IFN- γ -releasing cells were expressed as IFN- γ SFU/ 10^6 PBMC. Criteria to define a positive response included both ≥ 50 average spots/ 10^6 cells, a number of spots in the peptide-stimulated well ≥ 3 times greater than background, and a coefficient of variability < 70% in the triplicate wells.

* Letvin NL, Rao SS, Dang V, et al. No evidence for consistent virus-specific immunity in simian immunodeficiency virus-exposed, uninfected rhesus monkeys. *J Virol* 2007;81(22):12368-74.