Barcia et al., http://www.jem.org/cgi/content/full/jem.20060420/DC1

Immunofluorescence.
Immunocytochemical detection methods were optimized during preliminary experiments to achieve full and homogenous antibody penetration throughout the total thickness of vibratome sections. Adjacent 50-μm thick sections of each brain were pretreated with citrate buffer during 30 min at 60°C to increase antigen retrieval and penetration of the antibodies into the tissues. Sections were blocked with 1% Triton X-100 for 5 min and 3% normal horse serum in 0.1 M PBS, pH 7.4, for 60 min. The sections were incubated at room temperature for 48 h with primary antibodies combined. In the double or triple staining, the incubation with primary antibody was followed by 4 h of incubation with the appropriate secondary antibodies. Alexa 488–conjugated goat anti–rabbit antibody (1:1,000; Invitrogen), Alexa 594-conjugated goat anti–rat IgG antibody (1:1,000; Invitrogen), Alexa 594–conjugated goat anti–mouse IgG antibody (1:1,000; Invitrogen), Alexa 647–conjugated goat anti–guinea pig IgG antibody (1:1,000; Invitrogen), RRX goat anti–mouse IgG2a (1:500; Jackson ImmunoResearch Laboratories) or FITC-goat anti–mouse IgG1 (1:500; Jackson ImmunoResearch Laboratories). After washing, sections were incubated with DAPI solution for 30 min. Sections were washed, mounted, and examined with a microscopy for normal fluorescence (Carl Zeiss MicroImaging, Inc.) and analyzed with the confocal microscope (Leica).

DAB detection of immunocytochemistry.
Serial sections were used to detect transgene expression. Endogenous peroxidase activity was quenched with 0.3% H_2O_2 in PBS, nonspecific Fc binding sites were blocked with 10% horse serum, and sections were incubated for 48 h at room temperature with primary antibody diluted in PBS containing 1% horse serum and 0.5% Triton-X-100 (antibody solution). Sections were incubated for 4 h with biotin-conjugated appropriate secondary antibodies (DakoCytomation). Antibody binding was detected using avidin-biotin peroxidase with diaminobenzidine as chromogen, the glucose-oxidase system as a generator of H_2O_2, and intensification with Ni. These sections were mounted on gelatinized glass slides and were dehydrated before coverslipping.

Quantification of immunohistochemical staining.
Quantitative image analysis to estimate numbers of TK-, $CD8^+$-, and $CD4^+$-immunoreactive cells were performed with specific stereology software (StereoInvestigator) using a computer-assisted image analysis system with a Zeiss microscope connected to a digital camera through a zoom set (Carl Zeiss MicroImaging, Inc.), as described by us previously (39). The stereological estimation of the number of CD8 and TK immunoreactive cells was performed in the striatum and external capsule overlying the striatum, and anatomical locations were identified according to the atlas of the rat brain in 50-μm brain sections spaced at regular 250-μm intervals, as described in detail by us previously (Dewey, R.A., G. Morrissey, C.M. Cowsill, D. Stone, F. Bolognani, N.J. Dodd, T.D. Southgate, D. Klatzmann, H. Lassmann, M.G. Castro, and P.R. Lowenstein. 1999. *Nat. Med.* 5:1256–1263; Suwelack, D., A. Hurtado-Lorenzo, E. Millan, V. Gonzalez Nicolini, K. Wawrowsky, P. Lowenstein, and M.G. Castro. 2004. *Gene Ther.* 11:1742–1752). The estimation of the number of cells was performed using the principle of the optical fractionator. The number of cells was measured in 200-μm-sided dissectors covering the whole surface area of the analyzed regions (50 counting frames in each section). Results were expressed as absolute number of positive cells in the anatomical regions analyzed. Data are expressed as the means ± SEM.

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