

(GPGQVQLQ). Protein expression in M15pRep4 bacteria was induced with 0.5 mM IPTG.

Expression vectors. The complete ORF E8 of EHV-2 (viral DNA was a gift from A. J. Davison), ORF 159L of MCV (the plasmid was a gift from G. Darai) and ORF 71 of HVS were amplified by PCR and inserted in frame with an N-terminal Flag epitope into the *EcoRI* site of a vector derived from pCR-3 (Invitrogen). The Flag-E8-FLIP construct was subcloned into the pSR α puro vector (a gift from R. Sekaly) and then used to create stable puromycin-resistant transfectants of Jurkat and Raji cells. An expression vector for the Myc-tagged cytoplasmic domain of the murine CD95 receptor was generated by insertion of a PCR fragment corresponding to amino acids 166–306 in frame with an N-terminal Myc epitope into a pCR-3 derived vector. The expression vector for human TRAMP and FADD in pCR-3 has been previously described⁹. Human full-length CD95 was subcloned as a *HindIII/XbaI* fragment into pCR-3 and used for transfection of 293T cells.

Cell transfection. Stable puromycin-resistant transfectants of Jurkat and Raji cells were generated by electroporation of 8×10^6 cells in 800 μ l HeBS, mixed with 20 μ g SR α puro plasmid with or without Flag-E8 insert, at 250 V and 960 μ F. 48 h after transfection, cells were seeded at 10,000 cells per well in flat bottom 96-well plates under selection with 5 μ g ml⁻¹ puromycin (Sigma). 293T cells were seeded at $1-2 \times 10^6$ cells per 10-cm plate or $3-6 \times 10^5$ cells per 5-cm plate and transfected the next day by the calcium phosphate precipitation method. The precipitate was left on cells for 8 h, and cells were collected 26–30 h after transfection.

Cell lysis and co-immunoprecipitation. Cell lysis and co-immunoprecipitation of the various tagged proteins were carried out as described⁹. Jurkat and Raji clones or 293T cells were checked for protein expression by anti-tag western blot analysis of postnuclear cell lysates of equivalent protein content. Metabolic labelling of Raji cells with ³⁵S, anti-CD95 immunoprecipitations and 2D-gel electrophoresis were performed as previously described¹⁶. Analysis of DISC-associated FLICE activity was assessed as before¹⁷.

Apoptosis assays. The analysis of apoptosis induced by CD95L was carried out as follows: puromycin-resistant Jurkat clones ($\sim 3 \times 10^5$ cells per 500 μ l) were incubated for 3 h at 37 °C with 50 μ l supernatant from neuro-2a cells transfected with a murine CD95L expression vector or with control supernatant from cells transfected with mock vector²⁷. The susceptibility of Raji clones to anti-CD95-induced apoptosis was analysed by incubation of cells (5×10^3 cells ml⁻¹) with varying concentrations of anti-Apo-1 mAb¹⁶ in medium for 16 h at 37 °C. Apoptosis was measured by quantifying DNA fragmentation as previously described²⁸. Apoptosis of transiently transfected 293T cells was monitored by the cell-death detection ELISA (Boehringer Mannheim), which detects the presence of soluble histone–DNA complexes. The survival of E8-transfected and control Jurkat clones to TRAIL-induced cell death was tested by incubating cells at 50,000 cells per well in 100 μ l with the indicated concentrations of recombinant TRAIL and 1 μ g ml⁻¹ anti-Flag mAb for 20 h. Proliferating cells were subsequently quantified with the Celltiter 96 AQ proliferation assay (Promega) following the manufacturer's instructions.

Herpesvirus saimiri cultures and transcript analysis. The *in vitro* culture of virus and northern blot analysis of transcripts were carried out as described²². The effect of HVS infection on CD95-mediated cell death of owl monkey kidney (OMK) cells was assayed by seeding the cells at 10^4 cells per well in 96-well flat-bottomed plates. Two days later, half of the wells were infected at a multiplicity of infection of ~ 1 . Recombinant sCD95L (0.3 μ g ml⁻¹)⁹ was added together with enhancer (Alexis, San Diego) at different times after infection. Material was collected 20 h later and analysed for the presence of histone–DNA complexes as described.

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erratum

Ras signalling linked to the cell-cycle machinery by the retinoblastoma protein

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Due to an error in the reproduction process, the labelling in the right-hand panel of Fig. 5b of this letter was incorrect. The cell type involved is not *Rb*^{+/+} 3T3, but *Rb*^{-/-} 3T3, as indicated in the text and the figure legend.