

responsible for Vpr-induced cytotoxic effects, and only the ion channel-causing pathway was found to depend on the resting membrane potential in our previous work. The mechanism of the cytotoxic activity of the C-terminal portion of Vpr seems to be different, and our data suggest that glioma cells may be even more affected by the C-terminal Vpr cytotoxicity (Figure 2) than hippocampal neu-

rons (see Figure 8 in Piller *et al*, 1999). Therefore, in addition to the membrane potential requiring cytotoxic effect of Vpr, a second cytotoxic mechanism of the C-terminus appears to be more important in some cells. A wider range of different cell types needs to be screened for both the N-terminal and C-terminal cytotoxic effects to test this hypothesis.

References

- Huang MB, Weeks O, Zhao LJ, Saltarelli M, Bond VC (2000). Effects of extracellular human immunodeficiency virus type 1 Vpr protein in primary rat cortical cell cultures. *J NeuroVirology* **6**: 202–220.
- Piller SC, Gage PW, Jans P, Jans DA (1998). Extracellular HIV-1 virus protein R causes a large inward current and cell death in cultures hippocampal neurons: implications for AIDS pathology. *Proc Natl Acad Sci USA* **95**: 4595–4600.
- Piller SC, Ewart GD, Jans DA, Gage PW, Cox GB (1999). The amino-terminal region of Vpr from human immunodeficiency virus type 1 forms ion channels and kills neurons. *J Virology* **73**: 4230–4238.

Response

Effect of extracellular HIV-1 Vpr protein in vitro

VC Bond and M-B Huang

Department of Microbiology, Biochemistry, and Immunology, Morehouse School of Medicine, Atlanta, Georgia, USA

When identifying apoptosis, several assays, including the definitive morphological assay and fragmentation assay, should be used to avoid confusing apoptosis with necrosis (Grasl-Kraupp *et al*, 1995). Thus, although the data from our assays (Huang *et al*, 2000) suggest apoptosis, that from the definitive morphology and fragmentation assays suggested both apoptosis and necrosis were occurring in these cultures. We also would like to thank the author for providing new data clarifying their statement in Piller *et al* (1998). This evidence clearly shows that astrocytes are affected by the Vpr protein, causing cytotoxicity. In response to their statement concerning loss of cortical neurons, we were never able to identify apoptotic neurons in Vpr-treated cortical cultures as assayed by screening for NSE/TUNEL double-labeling (Grasl-Kraupp *et al*, 1995). We collected a body of unpublished data, including images from

Vpr-treated cultures exposed 24 h or more. We never observed cytotoxic cortical neurons as we did in the hippocampal cultures. This is also true for peripheral neurons (rat superior cervical ganglion neurons) which we have harvested and maintained in relatively pure culture. Additionally, based on the results presented in the author's letter, and assuming a similar cytotoxicity mechanism in hippocampal and cortical neurons, neuronal cytotoxicity in our cortical cultures should have (but did not) occurred early, 90 min, and peaked at 4 h (Grasl-Kraupp *et al*, 1995). Finally, the author's data in this letter and previous publications suggest a fast C-terminal cytotoxic mechanism that acts differentially on astrocytes (e.g., a membrane receptor not expressed in neurons), and a slower N-terminal mechanism that acts on both astrocytes and neurons (Piller *et al*, 1999).

References

- Grasl-Kraupp B, Ruttkay-Nedecky B, Koudelka H, Bukowska K, Bursch W, Schulte-Hermann R (1995). In situ detection of fragmented DNA (TUNEL assay) fails to discriminate among apoptosis, necrosis, and autolytic cell death: a cautionary note. *Hepatology* **21**: 1465–1468.
- Huang MB, Weeks O, Zhao LJ, Saltarelli M, Bond VC (2000). Effects of extracellular human immunodeficiency virus type 1 vpr protein in primary rat cortical cell cultures. *J NeuroVirol* **6**: 202–220.
- Piller SC, Gage PW, Jans P, Jans DA (1999). Extracellular HIV-1 virus protein R causes a large inward current and cell death in cultured hippocampal neurons: implications for AIDS pathology. *Proc Natl Acad Sci USA* **95**: 4595–4600.
- Piller SC, Ewart GD, Jans DA, Gage PW, Cox GB (1999). The amino-terminal region of Vpr from human immunodeficiency virus type 1 forms ion channels and kills neurons. *J Virol* **73**: 4230–4238.