Reply to Letter to the Editor

Response from authors RE: "Lack of association of herpesviruses with brain tumors"

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Similar to the paper by Cobbs *et al* (2002), we investigated the association of human cytomegalovirus (HCMV) infection with glioma. However, in accordance with recent publications of Lau *et al* (2005) and Sabatier *et al* (2005), we could not find hints for a role of this virus in glioma pathogenesis or maintenance of the transformed state of the tumor cells (Poltermann *et al*, 2006).

We totally agree that a very careful and sophisticated methodology is required to ascertain results revealing the presence of viral macromolecules in tumor tissue. This is one of the reasons why we tested not only brain tumor material but also blood samples of the same patients to exclude that HCMV-positive blood might contaminate tumor samples tested. In addition, in order to test the requirement of HCMV to maintain a tumorigenic phenotype, we tested the presence of HCMV in glioma cell lines and in cell cultures, freshly established from patient's tumor material: in none of the cases could HCMV DNA be detected. Reliability of polymerase chain reaction (PCR) was assured by the use of various protocols of sensitive nested PCRs, including the one described by Cobbs *et al* (2005), and always including sensitive positive and negative controls. In addition, more recently the group of B. Ehlers tested the samples described in our paper (and 92 additional glioma samples) for the presence of herpes virus sequences on a more general basis (cf. Ehlers *et al*, 1999, 2003). In 10% of the samples, herpes virus DNA sequences (e.g., human herpesvirus [HHV]-6, herpes simplex virus [HSV], Epstein-Barr virus [EBV]) could be found, but in none of the cases could HCMV DNA be detected (Ehlers *et al*, unpublished) despite the use of higher amounts of template DNA compared to those used in our published study.

According to our information, DNA extracted from frozen samples (−80°C, cf. Wilkens *et al*, 1994) is superior to paraffin-embedded tissue for sensitive PCR analysis.

Concerning immunohistochemistry, we used standard methods with 8-μm sections that had proven to routinely produce reliable results in other studies with other HCMV-infected tissues. For some of the glioma sections, we tested other antibodies in addition to those described in the paper, and also with prior pepsin digestion, without generating any HCMV protein detection (S. Poltermann, unpublished observation). Furthermore, we found that high antibody concentrations (e.g., 1:40, as suggested) were likely to produce false-positive results (signals in not HCMV-infected control cells). In contrast to the objection of Scheurer *et al*, in our hands, the use of Citra buffer seemed to increase sensitivity. However, because we did not have access to HCMV-infected brain tissue as the most appropriate positive control, we cannot totally exclude that our detection methods was not optimal for this kind of tissue.

Despite some possible weaknesses in methodology, the fact that neither in glioma cell lines nor in freshly obtained glioma cell cultures could HCMV macromolecules be detected is indicative of a lack of a role of this virus in glioma pathogenesis. Otherwise, all cells in culture should contain viral molecules that would be detected even with insensitive methods.

References

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