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Letter to the Editor

RE: "Lack of association of herpesviruses with brain tumors"

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In the April 2006 issue of the *Journal of NeuroVirology*, Poltermann *et al* (2006) report a lack of association between human cytomegalovirus (HCMV) and glioma. This subject is highly controversial, and the debate over the role of HCMV in glioma pathogenesis is ongoing. Given the fatal nature of these tumors, and the fact that very little is known about their etiology, extreme technical proficiency and scientific scrutiny must be used when examining potential causes. Several technical issues described in the article should be addressed to allow more accurate evaluation of the findings.

With regards to the immunohistochemistry protocol: First, the authors report using brain tumor sections 8 μm thick. Such thickness is typically used for surface staining and is known to be too thick for antigen-antibody binding. For optimal staining, a thickness of 6 μm is suggested (Cobbs *et al*, 2002). This allows complete deparaffinization, which is critical for proper enzyme digestion and antigen retrieval. Second, the authors fail to describe any postfixation of tissue sections with formalin prior to digestion, as well as any pepsin digestion before antigen retrieval. Formalin treatment (which stabilizes the antigen) is critical for epitope conditioning, and pepsin digestion is critical for HCMV detection because pepsin cleaves specific peptide residues that interfere with antigen-antibody binding. Third, the authors describe antigen retrieval using Citra buffer. This approach results in false-negative staining that could be overcome by combining both pepsin digestion and antigen retrieval (Polak *et al*, 2003) at 45°C to 50°C for 2.5 h; false-negative staining may result for conditions outside this range. Fourth, the antibody dilution used by the authors is very low (1:200 dilution). In our experience, a much higher dilution of 1:40 applied for at least 45 min is required for detection of active HCMV infection in sections of HIV-infected lung tissue (a reasonable nonbrain positive control). In the case of latent infection, a higher concentration (1:20) may be required (Arbustini *et al*, 1996). Fifth, the details of the detection system used, although critical, are poorly described.

With regards to the polymerase chain reaction (PCR) protocol: First, the use of DNA from frozen tumor samples for amplification of HCMV greatly increases the likelihood of obtaining false-negative results. This is due to the lack of preevaluation of the tissues before amplification and the possible presence of necrotic tissues in the tumor samples and relatively small amounts of actual tumor tissue. The use of paraffin-embedded sections is superior to frozen tissue because evaluation of the tumor using immunohistochemistry and *in situ* hybridization is possible. Second, the authors fail to indicate the concentration of the template DNA used for PCR-amplification reactions. They note that 2 μl of template DNA was used for the external PCR reaction, and 1 μl of product as template DNA for the internal reaction. In our laboratories, much higher DNA concentrations are required in order to detect low-copy HCMV infections.

We, therefore, strongly believe that this reported lack of association between HCMV and glioma is due to the seemingly minor but critical technical deficiencies.

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