Histopathological detection of owl's eye inclusions is still specific for cytomegalovirus in the era of human herpesviruses 6 and 7

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Abstract

***Background*—Cytomegalovirus (CMV) is the prototype member of the β-herpesvirinae, which can cause multiple organ dysfunction in the immunocompromised host. Human herpesvirus 6 (HHV-6) and HHV-7 are newer members of the β-herpesvirinae that can cause febrile illness in young children and are also possible pathogens in the immunocompromised patient.**

***Aim*—CMV is detected in histopathological sections by visualisation of owl's eye inclusion bodies. The aim of this study was to quantify the relation between CMV, HHV-6, and HHV-7 viral loads and the presence of owl's eye inclusions in histological sections.**

***Methods*—Histopathological examination of postmortem material and recording of owl's eye inclusion bodies were performed. CMV, HHV-6, and HHV-7 were detected by qualitative and quantitative polymerase chain reaction (PCR) from the same postmortem samples. Statistical analysis of the histopathological and PCR results was performed.**

***Results*—There was a significant association between the detection of owl's eye inclusion bodies and positive CMV PCR ($p < 0.001$); the median CMV viral load was significantly higher in samples that were positive for owl's eye inclusions ($p < 0.001$). No association was found between the presence of owl's eye inclusions and HHV-6 or HHV-7 positivity.**

***Conclusion*—Histological detection of owl's eye inclusion bodies is an insensitive but highly specific method for detecting CMV organ involvement. Owl's eye inclusion bodies are not associated with HHV-6 or HHV-7 infection.**

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Keywords: polymerase chain reaction; inclusion bodies; viral load

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Cytomegalovirus (CMV) is an important cause of multiple organ dysfunction in the immunocompromised host.[1] Patients can present with hepatitis, pneumonitis, ulceration of the oesophagus or colon, retinitis, or encephalitis. Organ involvement is routinely diagnosed by biopsy, with visualisation of owl's eye intranuclear inclusions in stained tissue sections.[2 3]

CMV (human herpesvirus 5) is the prototype member of the β-herpesvirinae, a subfamily of the herpesviridae.[4] In 1986 and 1990, respectively, two new herpesviruses were described and allocated to the β-herpesvirinae on the basis of their strong genetic relatedness to CMV; these viruses are termed human herpesvirus 6 (HHV-6)[5] and HHV-7.[6 7] HHV-6 and HHV-7 can each cause febrile illness in young children, including exanthem subitum,[8–10] and case reports suggest that, like CMV, HHV-6 may cause end organ disease in the immunocompromised host.[11] Other reports suggest that CMV associated disease might be increased in patients co-infected with HHV-7[12 13] or HHV-6.[14] It is not known whether HHV-6 and/or HHV-7 can produce owl's eye inclusions in vivo but, if they do, this could complicate the interpretation of a postulated associated between these other viruses and CMV associated disease.

We have developed quantitative competitive polymerase chain reaction (QCPCR) methods to detect each of these three β-herpesviruses[15–17] and quantify the viral load in biological samples, including tissue specimens.[18] In our study, we used these techniques to determine the sensitivity of histopathological visualisation of owl's eye inclusions to detect CMV infection and whether their presence is specific for CMV alone among the β-herpesvirinae.

Materials and methods

CLINICAL SAMPLES

To define the prevalence of CMV infection in patients with AIDS we prospectively collected multiple tissues from all such patients undergoing necropsies at this institution. For these clinicopathological studies, we aimed to collect up to 14 organs from each necropsy (lymph node, spleen, brain, lung, heart, kidney, adrenal, oesophagus, duodenum, colon, pancreas, liver, stomach, and salivary gland). A total of 139 organs were available from 11 unselected human immunodeficiency virus (HIV) positive patients (median, 14 organs/patient; range, 9–14). The median CD4 count at death was 10/mm^2 (range, 0–20). Nine patients had been prescribed zidovudine during their illness but all died before protease inhibitor drugs became available.[19]

HISTOPATHOLOGICAL EXAMINATION

The tissue samples were placed into buffered formalin during the course of a standard postmortem examination. After a minimum period of 48 hours in fixative, blocks were taken and processed through to paraffin wax. Sections were cut at 5 μm, stained with haematoxylin and eosin (Lillie's modification of Mayer's

Table 1 The number of organs that contained CMV, HHV-6, or HHV-7 DNA related to the presence of owl's eye inclusions

Owl's eye inclusions	*CMV DNA*			*HHV-6 DNA*			*HHV-7 DNA*		
	Pos	*Neg*	*Total*	*Pos*	*Neg*	*Total*	*Pos*	*Neg*	*Total*
Yes	19	0	19	13	6	19	9	10	19
No	75	45	120	87	33	120	83	37	120
Total	94	45	139	100	39	139	92	47	139
	p = 0.0004			p = 0.78			p = 0.07		

CMV, cytomegalovirus; HHV, human herpesvirus; neg, negative; pos, positive.

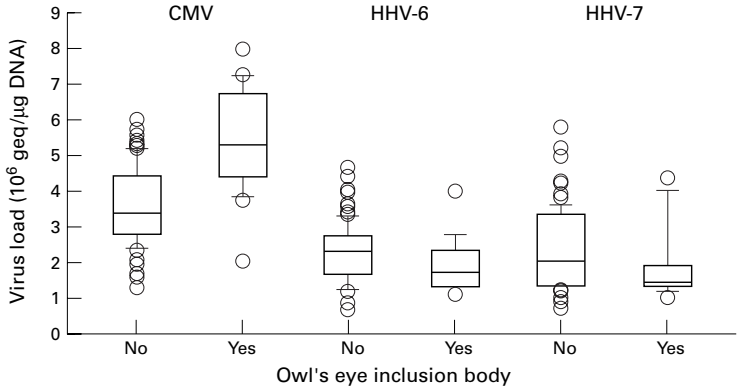

Figure 1 Box plots illustrating the relations between viral loads for three β-herpesviruses and the presence of owl's eye inclusions. The horizontal lines display the 10th, 25th, 50th (thick line), 75th, and 90th centiles; the boxes encompass 50% of the values; data points illustrate individual outlying values. CMV, cytomegalovirus; HHV, human herpesvirus.

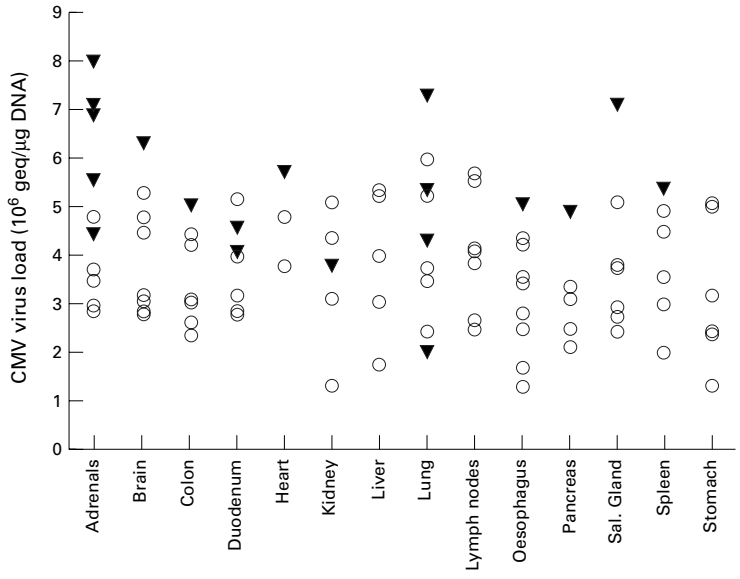

Figure 2 Association between the quantity of cytomegalovirus (CMV) DNA and the presence of owl's eye inclusions in particular organs. Closed triangle, CMV load associated with owl's eye inclusions; open circle, CMV load associated with negative staining for owl's eye inclusions.

haematoxylin), and examined. The presence of typical owl's eye inclusions was recorded as a positive finding. If the morphological features were considered inconclusive, immunohistochemical staining (Dako monoclonal antibody; Dako, Cambridge, UK) was carried out to provide confirmatory evidence. All of the sections were read by a single observer (JMcL).

EXTRACTION OF DNA

From each tissue, a block approximately $5 \times 5 \times 5$ mm was finely dissected and washed three times with sterile phosphate buffered saline. DNA was extracted from the tissue using the Promega Wizard DNA preparation kit (Promega, Southampton, UK), according to the manufacturer's instructions. The DNA was resuspended in water and 1 µg DNA used for all subsequent analyses (equivalent to ~ 1.5×10^5 diploid cells).

METHODS FOR PCR AND QCPCR

The methods used to detect CMV, HHV-6, and HHV-7, both qualitatively and quantitatively, have been described in detail elsewhere.[15–17] Briefly, the PCRs amplify genes UL55, U67, and U42 of CMV, HHV-6, and HHV-7, respectively. The sensitivity of the methods was comparable, with the ultimate sensitivity of detection of CMV being 5 geq/µg DNA, whereas the HHV-6 and HHV-7 QCPCR assays were capable of detecting 2 geq/µg DNA.

STATISTICAL METHODS

Contingency tables were constructed to show the relations between visualisation of inclusion bodies and the presence of each β-herpesvirus. The significance of any observed differences was assessed by means of the χ^2 test (or Fishers exact test where appropriate).

Among those samples that contained β-herpesvirus DNA detectable by PCR, we plotted the viral load (determined by QCPCR) for each virus according to whether or not owl's eye inclusions were seen. The significance of observed differences seen was examined by the student's *t* test.

Results

Owl's eye inclusions were seen in 19 of 139 tissues (13.5%). Inclusions were seen in organs from six of 11 patients. Inclusions were found on one or more occasion in 11 of 14 organs sampled (liver, stomach, and lymph node were negative in all cases).

Table 1 shows the results of qualitative PCR testing. There was a significant association between the detection of CMV by PCR and the presence of owl's eye inclusions ($p = 0.0004$). Of note, no inclusions were seen in tissues that were PCR negative. There was no association between the detection of HHV-6 and the presence of owl's eye inclusions, which were found in 13 of 100 (13%) HHV-6 PCR positive tissues compared with six of 39 (15%) HHV-6 PCR negative samples. For HHV-7, there was a trend for inclusions to be found less frequently in tissues that were PCR positive for HHV-7 (nine of 92; 10%) compared with those that were HHV-7 PCR negative (10 of 47; 21%). This difference was of borderline significance ($p = 0.07$).

We next analysed the relation between viral load for CMV, HHV-6, and HHV-7 in different organs and the visualisation of owl's eye inclusions in histological sections from these organs (fig 1).

The CMV viral load was significantly higher ($p < 0.001$; unpaired *t* test) in samples positive for owl's eye inclusions (mean viral load, 5.35×10^6geq/µg DNA; range, 2–7.95×10^6geq/µg DNA), compared with samples where no

owl's eye inclusions could be seen (mean viral load, 3.55×10^6geq/μg DNA; range, 1.3–5.99×10^6geq/μg DNA).

In contrast, no significant relation was found between the mean viral load for HHV-6 or HHV-7 from samples positive and negative for owl's eye inclusions. The mean viral load was slightly higher for HHV-6 (2.3×10^6geq/μg DNA; range, 0.7–4.6×10^6geq/μg DNA) and HHV-7 (2.3×10^6geq/μg DNA; range, 0.7–5.8×10^6geq/μg DNA) in tissue samples negative for owl's eye inclusions, compared with samples positive for owl's eye inclusions (HHV-6 mean viral load, 1.9×10^6geq/μg DNA; range, 1.1–3.9×10^6geq/μg DNA; HHV-7 median viral load, 1.8×10^6geq/μg DNA; range, 1–4.3×10^6geq/μg DNA).

Finally, we examined in detail the quantitative relation between CMV and the presence of inclusion bodies in particular organs (fig 2). Although the numbers of individual organs were small, in general, inclusions were seen in samples with high viral loads, with the exception of lung tissues.

Discussion

The results of our investigation confirm the high specificity of owl's eye inclusions for the diagnosis of CMV organ involvement. Specifically, the presence of inclusions correlated strongly with the detection of CMV DNA by PCR and did not correlate with the detection of HHV-6 or HHV-7 DNA by PCR. We conclude that the more recently described members of the β-herpesvirinae either do not produce owl's eye inclusions that can be confused with those of CMV, or that their incidence is so low as to make them undetectable by PCR. Although these results support the continued use of inclusion body detection in clinical practice,[20] it should be noted that the sensitivity of detecting inclusions is relatively low in that only 19 of 94 (20%) organs that contained detectable CMV DNA also had inclusions present. This observation confirms a report from 25 years ago that cell culture is approximately six times more sensitive than histology for detecting CMV in postmortem tissues.[21] Our QCPCR studies showed that inclusions were found significantly more frequently in tissues that contained high viral loads, which presumably reflects the difficulty of finding rare virus producing cells among a large background of uninfected cells.

This work is important because it investigates the specificity of detecting owl's eye inclusions, which is part of the internationally agreed case definition of CMV disease.[20] Recent reports suggest that CMV disease is more common among patients co-infected with HHV-7[12 13] or HHV-6.[14] One possible explanation for these observations could have been that HHV-6 and HHV-7 might themselves produce intranuclear inclusions and so lead to a false association with CMV disease. Our results show that this is not the case and so should facilitate future studies on the possible interactions between members of the β-herpesviruses in vivo.

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