Cytomegalovirus and human herpesviruses 6 and 7: Diseases and diagnosis in transplantation

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PD GRIFFITHS. Cytomegalovirus and human herpesviruses 6 and 7: Diseases and diagnosis in transplantation. Can J Infect Dis 1993;4(Suppl C):26C-32C. Similarities and differences in the epidemiology of cytomegalovirus (CMV), human herpesvirus 6 (HHV-6) and human herpesvirus 7 (HHV-7) infections are reviewed. Several distinct laboratory methods have been described for each virus. For CMV in immunocompromised patients, infection is best diagnosed by identifying active infection using routine surveillance cultures. Patients with active infection can then be entered into trials of suppressive therapy (where virus excretion is from the urine or saliva) or pre-emptive therapy (where excretion is detected systemically). For HHV-6 and HHV-7, only anecdotal cases of associations with disease in immunocompromised patients have been reported. Recommendations cannot therefore be made about appropriate diagnostic strategies or about treatment since it is not clear if these viruses are pathogens or passengers.

Key Words: *Diagnosis, Herpesvirus, Prognosis, Treatment*

Cytomégalovirus et herpèsvirus humains 6 et 7: maladies et diagnostic dans les cas de transplantation

RÉSUMÉ: Les ressemblances et les différences épidémiologiques des infections à cytomégalovirus (CMV) à herpèsvirus humain 6 (HHV-6) et à herpèsvirus humain 7 (HHV-7) sont passées en revue. Différentes méthodes de laboratoire ont été décrites pour chacun des virus. Pour le CMV chez les patients immunocompromis, l'infection est la mieux diagnostiquée par l'identification des réactions actives à l'aide de cultures de contrôle de routine. Les patients atteints d'une infection active peuvent ensuite être inscrits à des essais pour traitement suppresseur (quand l'excrétion virale se manifeste dans l'urine ou la salive) ou traitement préventif (quand l'excrétion systémique est décelée). Pour le HHV-6 et le HHV-7, seuls des cas anecdotiques d'associations pathologiques chez des patients immunocompromis ont été déclarés. Les recommandations doivent donc être formulées au sujet de stratégies diagnostiques appropriées ou au sujet de thérapeutiques, puisqu'on n'a pas déterminé si ces virus sont des pathogènes ou des passagers.

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HERPESVIRUSES ARE CLASSICAL OPPORTUNISTS IN THAT they reactivate when a patient is otherwise debilitated. It follows that herpesviruses can regularly be found in a variety of medical conditions which are immunocompromising or which require immunosuppressive drugs for their treatment. However, the mere presence of a herpesvirus in a patient does not necessarily mean that disease will result, and criteria which should be satisfied before a herpesvirus is associated with a particular disease have been reviewed. This paper summarizes how and why infections with two herpesviruses should be sought in transplant patients.

CYTOMEGALOVIRUS

As reviewed elsewhere in this issue, it is clear that cytomegalovirus (CMV) is the cause of several distinct clinical syndromes. Over the past decade, several research groups have contributed to the knowledge about the natural history of CMV infections in transplant patients. This information is an essential prelude to the design and conduct of controlled trials designed to interfere with natural history. Given this framework, the objectives of the laboratory are to guide clinical colleagues on potential management changes for individual patients under their care (Table 1). Each of these laboratory objectives will be considered in turn.

Pretransplant assessment: CMV antibodies of immunoglobulin (Ig) G class should be sought using one of a range of sensitive methods which are widely available. The presence of IgG antibodies in donors indicate that they are at risk of transmitting virus to the recipient (1). The presence of antibody in a recipient indicates that the patient is at risk of reactivating virus after transplantation (2). In the case of herpes simplex, the height of the pretransplant IgG level is generally predictive of future reactivation (3), but this is not true for CMV (4).

Post-transplant assessment: Once the patient is receiving immunosuppressive drugs, ability to mount a humoral immune response should not be relied upon for diagnostic purposes. Several methods exist for the detection of CMV. Cell culture remains the gold standard against which all newer methods must be compared. Nearly a decade ago, monoclonal antibodies reactive with the major immediate-early proteins of CMV were used in culture conformation to significantly shorten the time required for detection of CMV. This approach is termed DEAFF (detection of early antigen fluorescent foci) or shell vial technique (5,6). The sensitivity is lower than that of cell culture but can be significantly increased by centrifugation of clinical sample and cell culture before incubation. Monoclonal antibodies have also been used to detect CMV-specific antigens in leukocytes (polymorphonuclear and monocytes) from cytospin preparation of peripheral blood (7-9). Monoclonal antibodies used for this technique do not react with immediate-early antigens as originally thought, but recognize an early phosphoprotein of molecular weight ratio 65,000, termed the lower matrix protein (10). Finally, several methods for the detection of CMV by polymerase chain reaction (PCR) have been described (11-13).

TABLE 1
Cytomegalovirus laboratory objectives

Pretransplant	Detect latent cytomegalovirus in recipient and donor
	Advise on prophylaxis
Post-transplant	Monitor to detect active infection
	Provide prognostic information
	Advise on treatment strategies
	Detect resistant strains
	Advise on alternative treatments

The diagnosis of CMV infection after transplantation should ideally be made by collection of regular surveillance samples taken from time of transplant onwards. Most laboratories collect weekly urine, saliva and blood for this purpose and process them by one or more of the methods described above. Wherever possible, samples from a diseased organ such as liver biopsy or bronchoalveolar lavage should be collected to provide evidence of tissue infection. CMV disease should be classified according to a clinical scoring scheme (Table 2) pioneered by Plotkin et al (14,15) to facilitate evaluation of the protective responses of CMV vaccines.

Table 3 shows the use of this approach for three distinct groups of transplant patients under the care of clinical colleagues at the Royal Free Hospital School of Medicine. Samples from all patients were processed by cell culture and DEAFF. CMV infection is very common in transplant patients, but CMV disease does not occur in the same proportion of subgroups of patients. For example, in renal allograft patients, disease is largely restricted to those experiencing primary infection or reinfection from the donor organ (16). In contrast, bone marrow transplant patients have the most severe disease if their own virus is reactivated after transplantation (2). In our series, but not in others, a reduced incidence of disease was found if the marrow donor was seropositive (17). This may be due to the use of T cell depletion, which may remove the cells harbouring CMV infection, as well as those mediating graft-versus-host disease after transplantation. Under these circumstances, residual immunity in the donor may be adoptively transferred into the recipient (18). Since the donor marrow has been depleted of mature T cells, this suggests that the protective mechanism is non-T in origin, presumably B cell-mediated. The possibility of adoptive transfer of humoral immunity has been investigated by immunizing donors immediately before transplantation; the best results were obtained when the recipient as well as the donor were immunized (19).

The results from surveillance cultures can be analyzed in a different way to determine how much prognostic information they produce for the average patient.

TABLE 2
Scoring system for severity of cytomegalovirus disease

Clinical manifestations	Points
Fever (≥38.3°C)	
Mild (two to four days)	1
Moderate (five to 20 days)	1
Severe (≥21 days)	3
Leukopenia (<4x10^9/L)	1
Thrombocytopenia (<100x10^9/L)	1
Hepatitis	
Liver enzymes ≥2 times normal	1
Jaundice	3
Pneumonia associated with cytomegalovirus	
Infiltrate on x-ray	1
Infiltrate and symptoms	2
Ventilator support	3
Gastrointestinal bleeding or ulceration (biopsy proven)	3
Central nervous system changes	
Lethargy	1
Stupor	2
Coma	3
Renal insufficiency	
Creatinine level two to four times above best pretransplant level	1
Creatinine level more than four times above best pretransplant level	2
Nephrectomy, permanent dialysis	3
Arthritis or muscle wasting	2
Superinfection	
Bacterial, protozoal, fungal	3
Death	4

Adapted from Plotkin (14,15)

TABLE 3
CMV infection and disease at The Royal Free Hospital

Type of transplant	Pretransplant IgG		Number of patients	Number with CMV	
	R	D		Infection	Disease
Renal	–	–	21	0	0
	–	+	34	21 (62%)*	15 (44%)*
	+	–	51	19 (37%)*	3 (6%)
	+	+	71	49 (69%)*	18 (25%)*
Bone marrow	–	–	63	4 (6%)	2 (3%)
	–	+	23	6 (26%)*	1 (4%)
	+	–	25	12 (48%)*	11 (44%)*
	+	+	66	33 (50%)*	8 (12%)
Liver	–	–	4	1 (25%)	1 (25%)
	–	+	3	1 (33%)	1 (33%)
	+	–	18	2 (11%)	2 (11%)
	+	+	19	11 (58%)*	6 (32%)

**Significant difference from D–R– group; CMV Cytomegalovirus; D Donor; Ig Immunoglobulin; R Recipient*

Under these circumstances, the detection of CMV is assumed to be clinically useful only if it occurs in samples taken before the onset of disease in the patient. This approach was pioneered by the Seattle group, which showed an overall sensitivity of 69%, with a positive predictive value for CMV viremia of 60% using blood cultures maintained for five weeks (20). In an attempt to make prognostic information more readily available to physicians, we have used the DEAFF test, and found a lower sensitivity of about 50% but a positive predictive value of 64% (21). In renal transplant patients, we found a sensitivity of 79% and a positive predictive value of viremia of 46% (22), with a similar trend for liver transplant patients (23). Clearly, these results indicate that the sensitivity of surveillance cultures needs to be improved, but that viremia provides good prognostic value when it is detected. These results should therefore support the more widespread introduction of pre-emptive therapy where asymptomatic patients with CMV viremia are treated with an antiviral drug such as ganciclovir (24). In addition, the study should prompt more widespread evaluation of 'suppression', defined as administration of an antiviral drug to patients excreting only from peripheral sites of urine and/or saliva (25). Finally, these results should also stimulate the conduct of formal prognostic studies for newer methods such as polymerase chain reaction (PCR), which offer greatly increased sensitivity. Such studies must be conducted at each laboratory as variations in cell culture lines, techniques used, lengths of incubation, and anticoagulants used, can significantly alter the performance of a particular assay and the cell culture result with which it must be compared. Viremia should, therefore, not be seen as an all or none phenomenon but as a continuum which will be detected more readily by the most sensitive assays, but which will not necessarily be associated with severe disease in all cases. The ability to quantify PCR reactions should facilitate a greater refinement of the prognostic value of these assays (26).

Detection of resistant strains: Soon after ganciclovir became widely available for the treatment of CMV infections, strains of virus with reduced in vitro sensitivity to the drug were reported. For example, Erice et al (27) showed that strains of virus could acquire resistance to ganciclovir or could be selected during the course of an infection in vivo. The precise pathological potential of such resistant strains must be determined, but it seems likely that alternative therapy will be required for at least some patients, as has been described for herpes simplex (28).

One resistant strain has been studied in detail using marker rescue techniques, and resistance has been shown to map to two distinct genes: DNA polymerase and the product of the UL97 gene (29,30). By analogy with herpes simplex, the DNA polymerase mutant presumably has a reduced ability to recognize ganciclovir triphosphate. However, the UL97 gene provides a novel molecular target for CMV chemotherapy. The gene has been shown to code for a protein with a relative molecular mass of approximately 80,000, which is homologous

to protein kinases. A truncated protein with a relative molecular mass of approximately 39,000 was expressed in *Escherichia coli* and contained all of the predicted catalytic domains of the protein kinases. Extracts from the *E coli* were shown to phosphorylate ganciclovir and an antiserum reactive against UL97 was shown to neutralize the kinase activity.

An alternative drug for treating resistant strains is foscarnet. CMV strains resistant to ganciclovir are reported to be sensitive to this drug. In addition, the two drugs exhibit synergistic activity in vitro. They have been given together in patients, but there is at least one report of a strain of CMV resistant to both agents (31).

HUMAN HERPESVIRUSES 6 AND 7

Human herpesviruses 6 (HHV-6) and 7 (HHV-7) were not identified until 1986 and 1990, respectively, so much less information is available about their natural history than is the case for the other herpesviruses (32,33). Nevertheless, it appears that infection with both is commonly acquired in early childhood and that both viruses are frequently excreted in saliva (34-37). This is supported by the results of in situ hybridization and immunocytochemistry of salivary glands (38). One report describes the isolation of HHV-7 only from the saliva of adults, although this virus activated HHV-6 from the donor lymphocytes used for culture which became dominant subsequently (39). Most infected individuals exhibit no symptoms, suggesting that the natural history of these viruses resembles that of CMV or Epstein-Barr virus (EBV) rather than varicella-zoster virus (VZV). Molecular analyses of the genome of HHV-6 suggest that it is genetically related to CMV, with conserved blocks of genes colinear with their CMV counterparts (40). A map of restriction enzyme sites has been published (41).

HHV-6 has been shown to be the cause of exanthema subitum, a mild, self-limiting common childhood illness (42). Exanthema subitum was shown in 1950 to be transmissible to a six-month-old child and to macaques (43). In addition, case reports have associated HHV-6 with a variety of conditions. These are ranked in Table 4, from those which are probably caused by the virus to those which are purely speculative. This speculation includes the suggestion that HHV-6 could act as a cofactor to increase the rate at which acquired immunodeficiency syndrome (AIDS) develops following infection with human immunodeficiency virus (HIV). This is based upon in vitro experiments which show that HHV-6 can increase HIV replication in co-infected cells by stimulating transcriptional factors (44), or by transactivation (45,46), although this activation is dependent upon the reporter gene used (47). HHV-6 can up-regulate the CD4 molecule in CD8 cells to render them susceptible to HIV infection (48). However, another report shows that HHV-6 inhibits HIV replication in cell culture (49), while one serological clinical study does not support the hypothesis that HHV-6 adversely affects progression of HIV infection (50), although another does (51).

TABLE 4
Conditions associated with human herpesvirus 6 infection

	References
Exanthema subitum, including complications:	
mononucleosis	
leukopenia/thrombocytopenia	
hepatosplenomegaly	72
meningitis	73
encephalopathy	
fever	
Likely associations	
Meningitis	
Lymphadenopathy	
Mononucleosis	
Hepatitis	74
Pneumonitis	75,76
Suppression of marrow function	77
Speculative associations	
Post-viral fatigue syndrome	78-80
Cofactor for human immunodeficiency virus	This study
Lymphomas	57
Guillain-Barré syndrome	81
Collagen diseases	82,83
Intussusception	84

At present, there are no diseases associated with HHV-7 infection. Seroconversion to HHV-7 in a small number of healthy children appears to occur from the second year of life onwards (39).

Diagnosis of infection: Virus isolation has so far been the mainstay of diagnosis of this new herpesvirus (52,53). Immediate-early antigens have been identified and could be used for rapid viral diagnosis (54). Several PCR methods have been described (36,55-57). It is hoped that they, in conjunction with virus isolation, will be evaluated critically, as was described earlier for CMV, to help determine the true clinical significance of this virus (58).

In addition, some authors have begun to type strains of virus using slot-blot, restriction enzyme analysis, or PCR with sequencing of amplimers (59). Whether the strains associated with exanthema subitum should be differentiated taxonomically from the remaining strains is controversial (60).

The first serological tests used immunofluorescence, either using antihuman conjugates or using anticomplement immunofluorescence (61,62). Given the genetic relationship with CMV, the fact that the virus may activate host cell proteins (63) and the possibility that it may encode an Fc receptor, it has been difficult for investigators to determine uniequivocally a true cut-off between seropositive and seronegative. When serum dilutions of 1:80 are used in indirect immunofluorescence, then, typically, 80% of the population are shown

to be seropositive. Serological responses in patients have been reported during CMV infections, and vice versa. Whether this results from antigenic cross-reaction or co-reactivation of both herpesviruses in immunocompromized patients, is unknown (64-66). Other serological methods have been described, including enzyme immunoassay (67,68), IgM determination (69,70), Western blotting (51) and radioimmunoprecipitation (71), but their specificity and sensitivity remain to be determined.

CONCLUSION

Work towards the laboratory diagnosis of HHV-6 is recapitulating that done decades earlier for CMV. It is hoped that progress will be more rapid, however, since we now have the benefit of using molecular biological approaches. When using these techniques, investigators should not ignore the complex natural history of herpesviruses and so will use the methods to differentiate clearly between clinical associations which are causal and those which are merely casual.

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