Oral Diseases (2007) 13, 329–334. doi:10.1111/j.1601-0825.2006.01294.x

http://www.blackwellmunksgaard.com

ORIGINAL ARTICLE

Detection of human herpesvirus 6 in patients with oral chronic graft-*vs*-host disease following allogeneic progenitor cell transplantation

CM Pereira[1], OP de Almeida[1], MEP Corrêa[2], FF Costa[2], CA de Souza[2], ML Barjas-Castro[2]

[1]*Oral Semiology and Oral Pathology, School of Dentistry of Piracicaba, State University of Campinas, São Paulo;* [2]*Hemocentro UNICAMP, State University of Campinas, São Paulo, Brazil*

INTRODUCTION: Chronic graft-*vs*-host disease (cGVHD) is a major cause of morbidity in long-term survivors of allogeneic hematopoietic progenitor cell transplantation. Herpesviruses are involved in the occurrence and progression of various oral diseases.

AIM: The aim of this study was to investigate the role of human herpesvirus 6 (HHV6) in patients with oral manifestations of cGVHD.

MATERIALS AND METHODS: Peripheral blood and oral fluids (whole saliva, gingival crevicular fluid and parotid gland saliva) from 19 cGVHD patients, and 28 blood donors were examined for HHV6. Oral tissue samples were collected from 12 cGVHD patients and 12 healthy individuals. Nested polymerase chain reaction was employed to identify the HHV6.

RESULTS AND CONCLUSION: The virus was detected in whole saliva in 13 cGVHD patients (68%) and in 19 blood donors (67%). HHV6 was not identified in any of the gingival crevicular fluid and parotid gland saliva samples in cGVHD patients. In the control group 14.3% of both, four gingival crevicular fluid and four parotid gland saliva samples were positive. Two oral tissue samples of cGVHD patients were positive for HHV6. These results indicate that patients with oral manifestations of cGVHD and healthy individuals present high and similar incidence of HHV6 in blood and oral fluids. These data do not support the importance of HHV6 in oral lesions of cGVHD.

Oral Diseases (2007) **13**, 329–334

Keywords: human herpesvirus 6; graft-*vs*-host disease; hematopoietic progenitor cell transplantation; saliva; mouth

Correspondence: Claudio Maranhão Pereira, Hemocentro UNICAMP/State University of Campinas, Unicamp, Rua Carlos Chagas, 480, PO Box 6198/CEP: 13083-970, Campinas, SP, Brazil. Tel: 55 19 37888734, Fax: 55 19 32891089, E-mail: claudiomaranhao@hotmail.com
Received 23 October 2005; revised 19 January 2006, 31 March 2006; accepted 18 May 2006

Introduction

Allogeneic bone marrow transplantation (BMT) has been used as a therapeutic modality for patients with malignant neoplasms, immunodeficiency diseases and syndromes of marrow failure. Graft-*vs*-host disease (GVHD) is a common complication in hematopoietic progenitor cell transplant patients (Sullivan, 1986). Acute GVHD usually appears within the first 3 months following transplantation and is diagnosed in 30–50% of hematopoietic cell transplant recipients (Couriel *et al*, 2004). Chronic GVHD (cGVHD) is developed 3 or more months after hematopoietic cell transplantation, affecting 60–80% of the long-term survivors (Sullivan, 1986).

Oral manifestations have been described in approximately 80% of the patients with cGVHD. Oral lesions closely resemble those seen in a variety of autoimmune connective tissue diseases (Rodu and Gockerman, 1983; Schubert and Sullivan, 1990; Hiroki *et al*, 1994; Nicolatou-Galitis *et al*, 2001). Lichenoid reactions typically observed as white striae are the most distinctive oral lesions and occur in 80–100% of patients with cGVHD. Salivary glands can also be affected (Hiroki *et al*, 1994; Nagler *et al*, 1996; Nicolatou-Galitis *et al*, 2001).

Human herpesvirus 6 (HHV6) is one of the eight types of viruses from the Herpesviridae family and can be separated into two closely related and distinct groups, designated variants A and B. Primary infection with HHV6 variant B causes exanthem subitum in infants, while the clinical features of variant A infection remain poorly defined. HHV6 remains latent after primary infection in selected anatomical sites, such as salivary glands (Levy *et al*, 1990; Cone *et al*, 1993), mononuclear cells (Kondo *et al*, 1991), genital secretions (Prober, 2005) and the central nervous system (Cuomo *et al*, 2001). Reactivation or re-infection may occur, particularly in patients with immune deficiency. In hematopoietic cell recipient patients, the virus might enhance immune system reactivity or alter the antigenicity of the host tissue, thereby leading to an increased incidence of GVHD (Wilborn *et al*, 1994).

Saliva has been considered an important means for HHV6 transmission, but the main source of the virus found in the oral cavity is not yet clear (Suga *et al*, 1992; Pereira *et al*, 2004). HHV6 can be detected in periodontal diseases, recurrent aphthous ulcerations, leukoplakia, epithelial tumors and oral lichen planus. The presence of the virus, and its role in the pathogenesis of oral diseases, however, has yet to be fully delineated (Yadav *et al*, 1997; Brice *et al*, 2000; Cassai *et al*, 2003).

The aim of this study was to investigate the role of HHV6 in blood, oral fluids and buccal mucosa samples of patients with oral manifestations of cGVHD.

Patients and methods

Nineteen patients with cGVHD were subjected to medical and dental evaluations. The presence of lichenoid lesions (white striae, atrophic-ulcers, hyperkeratosis and mixed forms) in clinical examination was considered oral manifestations of GVHD (Franca *et al*, 2001; Ratanatharathorn *et al*, 2001; Dominguez Reyes *et al*, 2003). Oral tissue samples (oral mucosa and minor salivary gland) were used for diagnosis and histopathological staging of cGVHD according to Horn *et al* (1995).

Xerostomia was clinically evaluated as mild (reduction of the sublingual salivary pool, adherence of the wood spatula to the cheek mucosa) and severe (absence of the sublingual salivary pool, higher adherence of the wood spatula to the cheek mucosa, dry lips and tongue snap). Data regarding the graft type (bone marrow or peripheral blood cell source), organs involved by cGVHD and drugs received at the moment of sample collection were recorded. Samples of whole saliva, parotid gland saliva and gingival crevicular fluid were collected from 17 patients with cGVHD. It was impossible to collect oral fluids in two patients as a result of severe hyposalivation. Biopsies of oral tissue of the lower lip from 12 patients were also used to detect HHV6. The material was fixed in 10% formalin and embedded in paraffin (Dressler *et al*, 1999).

Control groups

Twenty-eight blood donors were examined for the presence of HHV6 in peripheral blood, whole saliva, crevicular fluid and parotid gland saliva. Normal oral tissue samples fixed in 10% formalin and embedded in paraffin were obtained from 12 individuals with mucoceles of the lower lip, who underwent surgical treatment. All the individuals of the control group were healthy, without any other oral lesions.

This study followed the guidelines described by the Brazilian Medical Research Center and received approval from the Research Ethics Committee of the State University of Campinas (UNICAMP).

Peripheral blood, oral fluids and buccal mucosa analysis

Peripheral blood (10 ml) was collected in a tube with anticoagulant (EDTA) and submitted to leukocyte and neutrophil counting (Cell-Dyn 3500; Abbott, Amstelveen, The Netherlands). DNA extraction from peripheral blood mononuclear cells was performed as described by Wilborn *et al* (1994).

Unstimulated whole saliva (2.0 ml) was collected in sterile plastic tubes and 75 μl of parotid gland saliva and gingival crevicular fluid were collected using sterile paper endodontic cones (Pereira *et al*, 2004). The samples underwent DNA extraction as described by Cone *et al* (1993).

Genomic DNA was extracted from 5-μm thick sections of formalin-fixed paraffin-embedded tissue as described by Shibata (1994). Briefly, two sections that underwent paraffin extraction with xylene, were washed twice with ethanol (100%), and vacuum dried. This was followed by digestion for 4 h at 55°C, with 400 μg ml^{-1} proteinase K, 100 mM Tris-HCl, 4 mM EDTA (pH 8.0), and boiled for 10 min to inactivate the enzyme. The samples were stored at −20°C until analysis.

Human herpesvirus 6 nested polymerase chain reaction was carried out using two sets of nested primers previously described (Secchiero *et al*, 1995). In order to confirm the presence of intact DNA in the samples a beta-globin primer pair was used as control (Cone *et al*, 1993). Positive and negative DNA control samples, as well as DNA-free samples were included in each set of reactions. Statistical analysis was performed using Fisher test comparing the incidence of HHV6 in both groups, control and cGVHD patients. Statistical significance was assigned at $P = 0.05$.

Results

Fourteen (73.7%) patients of the cGVHD group were male and five (26.3%) were female, with an age range of 19–63 years (mean 39). Twelve (63.1%) patients had chronic myeloid leukemia, three (15.8%) severe aplastic anemia, two (10.5%) acute myeloid leukemia, one (5.3%) acute lymphocytic leukemia and one (5.3%) multiple myeloma. Thirteen (68.4%) patients received bone marrow cells (BM) and 6 (31.6%) peripheral hematopoietic progenitor cells (PHPC).

Thirteen (68.4%) presented extensive and six (31.6%) limited cGVHD. Among six limited cGVHD, one involved the skin and five the buccal mucosa. Three (15.8%) of 19 patients had a previous history of acute GVHD involving the buccal mucosa. Nine (47.4%) patients presented xerostomia, in four this was severe and in five this was mild (Table 1).

At the time of sample collection, 17 patients were receiving cyclosporin A (10 mg kg^{-1} day^{-1}) and 13 were receiving prednisone (1 mg kg^{-1} day^{-1}). Four patients were taking acyclovir (800 mg day^{-1}), three for herpes simplex infection and one for varicella zoster.

Six patients presented the lichenoid form of cGVHD in the buccal mucosa, six the atrophic-ulcerative form, two the hyperkeratotic form and three the mixed form. Two patients did not present oral lesions at the time of clinical examination but did present a histopatological diagnosis of cGVHD involving the oral tissue and minor salivary gland (Table 1).

Table 1 Oral histopathological cGVHD grade, cell source of hematopoietic progenitor cell transplantation, leukocyte and neutrophil counts and examination for the presence of HHV6 in peripheral blood and oral fluids of 19 patients with cGVHD

								HHV6	
N	*Organ affected*	*Cell source*	*Xerostomia*	*Oral lesion cGVHD*	*Oral cGVHD grade*	*Leukocyte* ($\times 10^3\ \mu l^{-1}$)	*Neutrophil* ($\times 10^3\ \mu l^{-1}$)	*Whole saliva*	*Blood*
1	Mouth, eyes, skin, lung	BMC	Severe	Atrophic-ulcerative widespread	2	7.0	4.6	+	−
2	Mouth, skin, liver	BMC	Absent	Atrophic-ulcerative buccal mucosa	3	7.5	4.7	+	+
3	Mouth, liver	PHPC	Mild	Atrophic-ulcerative widespread	2	4.4	2.5	+	−
4	Mouth	BMC	Severe	Lichenoid widespread	2	3.0	1.9	+	+
5	Mouth	BMC	Mild	Atrophic-ulcerative widespread	1	3.6	2.1	+	+
6	Mouth, eyes	BMC	Absent	Hyperkeratosis tongue	2	6.3	5.3	−	−
7	Mouth, skin	BMC	Severe	Lichenoid widespread	2	6.0	3.9	−	+
8	Mouth	BMC	Absent	Mixing form	3	2.6	1.8	+	+
9	Mouth, skin	PHPC	Absent	Atrophic-ulcerative widespread	3	11.0	7.5	−	−
10	Mouth, lung	BMC	Severe	Mixing form	2	5.3	3.2	−	−
11	Mouth, skin, liver, kidney	PHPC	Absent	Hyperkeratosis buccal mucosa	2	5.8	3.0	+	+
12	Mouth, skin, eyes	PHPC	Mild	Lichenoid widespread	4	11.5	8.4	+	+
13	Mouth	BMC	Absent	Without lesion	1	5.0	2.6	+	−
14	Mouth, skin	BMC	Mild	Lichenoid widespread	1	2.7	1.9	−	−
15	Skin	BMC	Absent	Without lesion	2	4.5	2.0	+	+
16	Mouth, skin	PHPC	Absent	Lichenoid widespread	1	4.4	2.7	+	+
17	Mouth, skin, liver	BMC	Absent	Mixing form	3	6.8	4.6	+	−
18	Mouth	BMC	Absent	Atrophic-ulcerative widespread	1	2.6	1.8	+	−
19	Mouth, skin, eyes, liver	PHPC	Mild	Lichenoid widespread	2	11.7	3.1	−	+

cGVHD, chronic graft-*vs*-host disease; HHV6, human herpesvirus 6; BMC, bone marrow cells; PHPC, peripheral hematopoietic progenitor cells.

Leukocytes above $11.0 \times 10^3\ \mu l^{-1}$ were observed in two patients and below $4.5 \times 10^3\ \mu l^{-1}$ in seven patients of 19 with cGVHD (Table 1).

HHV6 PCR analysis

The beta-globin DNA gene was clearly amplified in all the samples analyzed in both the groups, control and cGVHD patients. Tests for HHV6 in the oral fluids of cGVHD patients were performed in 17 of 19 patients. Two individuals presented severe hyposalivation and collection of saliva was impossible (Table 1). Twenty-eight blood donors formed the control group of which 20 (71.4%) were male and eight (28.5%) were female, with the age range of 20–60 years, mean of 39.6 years.

Table 2 shows the results of HHV6 in cGVHD patients and control group. Two (16.6%) patients with cGVHD presented positive results for HHV6 in oral tissue biopsies, and all the individuals from the control group were negative. Both positive patients presented oral atrophic ulcerative widespread lesions. Statistical analysis comparing the incidence of HHV6 in both the groups, control and cGVHD patients, showed $P = 0.74$.

Discussion

Patients undergoing chemotherapy and hematopoietic cell transplantation are susceptible to infections with several opportunistic pathogens (Wang *et al*, 1996). Reactivation of HHV6 following hematopoietic cell transplantation was first reported by Yoshikawa *et al* (1991), and clinical manifestations of this reactivation have been reported from then on, including pneumonitis, encephalitis and GVHD (Singh and Carrigan, 1996; Johnston *et al*, 1999; Zerr *et al*, 2001). On the other hand, the relationship between increased HHV6 replication in blood and clinically recognizable disease of these patients has generally been difficult to establish (Yoshikawa, 2004).

We studied patients with cGVHD and the results demonstrated that 15 (78.9%) of 19 patients were positive for HHV6 (13 oral fluids and two peripheral blood). The *P*-value showed that there was no statistical difference in the incidence of positive HHV6 samples between cGVHD patients and healthy individuals ($P = 0.74$). HHV6 may be detected in human saliva, but the percentage of the involved population is variable. (Levy *et al*, 1990; Cone *et al*, 1993). The reasons for this variation are yet unclear. Some primers used for HHV6 may react with HHV7 (Di Luca *et al*, 1995); however, we used primers derived from a highly conserved sequence of the HHV6 genome.

Gingival crevicular fluid is the main source of leukocytes found in saliva (Lantzman and Michman, 1970). Thirteen (68.4%) samples of whole saliva from patients with cGVHD were positive for HHV6 while all the samples of gingival crevicular fluid and of parotid gland saliva were negative. Nevertheless, all the samples of gingival crevicular fluid and parotid saliva were positive for the beta-globin gene amplification. These data suggest that gingival crevicular fluid and parotid gland saliva were not the principal sources of the HHV6 found in whole saliva.

All the patients had leukocyte numbers normal or close to the normal limit. Nine patients had neutrophil counts below the normal limit $3.0 \times 10^3\ \mu l^{-1}$ (Ryan, 2001); nevertheless no one had values below

Table 2 HHV6 detected by polymerase chain reaction in peripheral blood, whole saliva, parotid gland saliva and gingival crevicular fluid from 19 cGVHD patients and 28 healthy individuals

HHV6 investigation	*Control group (n = 28)*	*cGVHD (n = 19)*
HHV6 positive, *n* (%)	20	15
Peripheral blood	0	2 (10.5)
Peripheral blood, whole saliva	8 (28.6)	8 (42.1)
Peripheral blood, gingival crevicular fluid	1 (3.6)	0
Peripheral blood, whole saliva, parotid gland saliva	1 (3.6)	0
Peripheral blood, whole saliva, gingival crevicular fluid, parotid gland saliva	1 (3.6)	0
Whole saliva	6 (21.4)	5 (26.3)
Whole saliva, gingival crevicular fluid	1 (3.6)	0
Whole saliva, parotid gland saliva	1 (3.6)	0
Whole saliva, gingival crevicular fluid, parotid gland saliva	1 (3.6)	0
HHV6 negative, *n* (%)	8	4
Peripheral blood, oral fluids	8 (28.5)	2 (10.5)
Peripheral blood[a]		2 (10.5)
Total, *n* (%)	28 (100)	19 (100)

cGVHD, chronic graft-*vs*-host disease; HHV6, human herpesvirus 6.
Fisher test: $P = 0.74$.
[a]It was impossible to perform the study of HHV6 in oral fluid of two patients as a result of severe hyposalivation.

1.8×10^3 μl^{-1}, which represents a high risk of infections (Dale, 2001). Eight patients were positive for HHV6 investigation and their neutrophil numbers were below 3.0×10^3, however seven had cGVHD grade 1 or 2.

Epithelium of the salivary glands has been shown to be affected early in the course of cGVHD, with an incidence of 80–100%. Additionally, a direct correlation has been observed between the degree of hyposalivation and the severity of GVHD (Schubert and Sullivan, 1990; Hiroki *et al*, 1994; Nagler *et al*, 1996). Nine of our patients (47.3%) complained of xerostomia; however, only four of them were classified clinically as severe, and presented histopathologically oral cGVHD grade 2. The results did not suggest association between the clinically observed xerostomia and histopathological severity of oral cGVHD.

The influence of the graft type in HHV6 infection is still controversial. Maeda *et al* (1999) detected higher rates of HHV6 DNA in patients who had undergone allogeneic transplantation using bone marrow cells, compared with PHPC. However, Ljungman *et al* (2000) described that the graft type did not influence the HHV6 viral load. In our study, 10 of 13 and five of six patients were positive for HHV6 after BMT and PHPC transplantation, respectively. In spite of the small number of cases studied, our results agree with those of Ljungman *et al* (2000) showing that the cell source did not influence the incidence of HHV6 infection.

In our study four patients were receiving acyclovir during sample collection. These patients presented oral manifestations of cGVHD and were positive for HHV6 in oral fluids. These results support other findings reported in the literature, where HHV6 is reported to be less sensitive to acyclovir than ganciclovir and foscarnet (Singh and Carrigan, 1996; Zerr *et al*, 2001).

Lichenoid lesions with predominant reticular and papular forms are the most frequent cGVHD oral manifestations occurring in 80–100% of these patients. Ulcerative-atrophic and hyperkeratotic forms are less common (Rodu and Gockerman, 1983; Schubert and Sullivan, 1990; Hiroki *et al*, 1994; Nicolatou-Galitis *et al*, 2001; Dominguez Reyes *et al*, 2003). In our study, the incidence of lichenoid lesions was similar to the other forms of oral lesions, particularly ulcerative-atrophic. The data also suggested that there was no association between the type of oral lesions and the detection of the virus.

Although PCR is extremely effective with pure and fresh biological specimens, its success with complex biological samples such as paraffin-embedded tissues is variable (Giroti and Kashyap, 1998). The efficiency of PCR amplification decreases in accordance with an increase in fixation time and the acidification of formalin with formic acid (Inoue *et al*, 1996). The main obstacle in preparing suitable DNA for PCR amplification is the removal of paraffin wax and purification (Coombs *et al*, 1999). Nevertheless, this methodology has been used successfully, and it is important to improve the technique as many valuable samples are routinely embedded in paraffin for diagnosis.

In our study, only two patients with cGVHD were positive for HHV6 in the oral mucosa and minor salivary glands. Both patients presented oral atrophic-ulcerative widespread lesions. In spite of the detection of HHV6 in these two patients, it was difficult to associate it with the clinical manifestations. It was also impossible to discard that the small number of positive cases was a result of the presence of the PCR inhibitor in the samples.

In conclusion, the results of this work showed a high and similar presence of HHV6 in the oral fluids of both the cGVHD patients and healthy individuals. We could not confirm the presence of HHV6 and its role in the pathogenesis of oral lesions associated with cGVHD. Further studies are necessary to verify the role of HHV6 in the oral manifestations of cGVHD.

Acknowledgements

This work was supported by FAPESP – Fundação de Amparo a Pesquisa do Estado de São Paulo and CAPES.

References

Brice SL, Cook D, Leahy M, Huff JC, Weston WL (2000). Examination of the oral mucosa and peripheral blood cells of patients with recurrent aphthous ulceration for human herpesvirus DNA. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **89:** 193–198.

Cassai E, Galvan M, Trombelli L, Rotola A (2003). HHV-6, HHV-7, HHV-8 in gingival biopsies from chronic adult periodontitis patients. A case-control study. *J Clin Periodontol* **30:** 184–191.

Cone RW, Huang ML, Ashley R, Corey L (1993). Human herpesvirus 6 DNA in peripheral blood cells and saliva from immunocompetent individuals. *Clin Microbiol* **31:** 1262–1267.

Coombs NJ, Gough AC, Primrose JN (1999). Optimisation of DNA and RNA extraction from archival formalin-fixed tissue. *Nucleic Acids Res* **27:** e12 (i–iii).

Couriel D, Caldera H, Champlin R, Komanduri K (2004). Acute graft-versus-host disease: pathophysiology, clinical manifestations, and management. *Cancer* **101:** 1936–1946.

Cuomo L, Trivedi P, Cardillo MR *et al* (2001). Human herpesvirus 6 infection in neoplasic and normal brain tissue. *J Med Virol* **63:** 45–51.

Dale DC (2001). Neutropenia and neutrophilia. In: Beutler E, Lichtman MA, Coller BS Kipps TJ, Seligsohn U, eds. *Williams hematology*, 6th edn. Medical Publishing Division, McGraw-Hill: New York, pp. 823–834.

Di Luca D, Mirandola P, Ravaioli T *et al* (1995). Human herpesvirus 6 and 7 in salivary gland and human immunodeficiency virus positive individuals. *J Med Virol* **45:** 426–465.

Dominguez Reyes A, Aznar Martin T, Barberia Leache E, Cabrera Suarez E (2003). Oral manifestations of graft versus host disease. Case report. *Med Oral* **8:** 361–365.

Dressler LG, Geradts J, Burroughs M, Cowan D, Millikan RC, Newman B (1999). Policy guidelines for the utilization of formalin-fixed, paraffin-embedded tissue sections: the UNC SPORE experience. University of North Carolina Specialized Program of Research Excellence. *Breast Cancer Res Treat* **58:** 31–39.

Franca CM, Domingues-Martins M, Volpe A, Pallotta Filho RS, Soares de Araujo N (2001). Severe oral manifestations of chronic graft-vs.-host disease. *J Am Dent Assoc* **132:** 1124–1127.

Giroti R, Kashyap VK (1998). Detection of the source of mislabeled biopsy tissue paraffin block and histopathological section on glass slide. *Diagn Mol Pathol* **7:** 331–334.

Hiroki A, Nakamura S, Shinohara M, Oka M (1994). Significance of oral examination in chronic graft-versus-host disease. *Oral Pathol Med* **23:** 209–215.

Horn TD, Rest EB, Mirenski Y, Corio RL, Zahurak ML, Vogelsang GB (1995). The significance of oral mucosa and salivary gland pathology after allogeneic bone marrow transplantation. *Arch Dermatol* **131:** 964–965.

Inoue T, Nabeshima K, Kataoka H, Koono M (1996). Feasibility of archival non-buffered formalin-fixed and paraffin-embedded tissues for PCR amplification: an analysis of resected gastric carcinoma. *Pathol Int* **46:** 997–1004.

Johnston RE, Geretti AM, Prentice HG *et al* (1999). HHV-6-related secondary graft failure following allogeneic bone marrow transplantation. *Br J Haematol* **105:** 1041–1043.

Kondo K, Kondo T, Okuno T, Takahashi M, Yamanishi K (1991). Latent human herpesvirus 6 infection of human monocytes/macrophages. *J Gen Virol* **72:** 1401–1408.

Lantzman E, Michman J (1970). Leukocyte counts in the saliva of adults before and after extraction of teeth. *Oral Surg Oral Med Oral Pathol* **30:** 766–773.

Levy JA, Ferro F, Greespan D, Lennette ET (1990). Frequent isolation of HHV6 from saliva and high seroprevalence of the virus in the population. *Lancet* **335:** 1047–1050.

Ljungman P, Wang FZ, Clark DA *et al* (2000). High levels of human herpesvirus 6 DNA in peripheral blood leucocytes are correlated to platelet engraftment and disease in allogeneic stem cell transplant patients. *Br J Haematol* **111:** 774–781.

Maeda Y, Teshima T, Yamada M *et al* (1999). Monitoring of human herpesviruses after allogeneic peripheral blood stem cell transplantation and bone marrow transplantation. *Br J Haematol* **105:** 295–302.

Nagler R, Marmary Y, Krausz Y, Chisin R, Markitziu A, Nagler A (1996). Major salivary gland dysfunction in human acute and chronic graft-versus-host disease (GVHD). *Bone Marrow Transplant* **17:** 219–224.

Nicolatou-Galitis O, Kitra V, Van Vliet-Constantinidou C *et al* (2001). The oral manifestations of chronic graft-versus-host disease (cGVHD) in pediatric allogeneic bone marrow transplant recipients. *J Oral Pathol Med* **30:** 148–153.

Pereira CM, Gasparetto PF, Côrrea MEP, Costa FF, Almeida OP, Barjas-Castro ML (2004). Human herpesvirus 6 in oral fluids from healthy individuals. *Arch Oral Biol* **49:** 1043–1046.

Prober C (2005). Sixth disease and the ubiquity of human herpesviruses. *N Engl J Med* **352:** 753–755.

Ratanatharathorn V, Ayash L, Lazarus HM, Fu J, Uberti JP (2001). Chronic graft-versus-host disease: clinical manifestation and therapy. *Bone Marrow Transplant* **28:** 121–129.

Rodu B, Gockerman JP (1983). Oral manifestations of the chronic graft-v-host reaction. *JAMA* **249:** 504–507.

Ryan DH (2001). Examination of the blood. In: Beutler E, Lichtman MA, Coller BS Kipps TJ, Seligsohn U, eds. *Williams hematology*, 6th edn. Medical Publishing Division, McGraw-Hill: New York, pp. 9–16.

Schubert MM, Sullivan KM (1990). Recognition, incidence, and management of oral graft-versus-host disease. *NCI Monogr* **9:** 135–143.

Secchiero P, Carrigan DR, Asano Y *et al* (1995). Detection of human herpesvirus 6 in plasma of children with primary infection and immunosuppressed patients by polymerase chain reaction. *J Infect Dis* **171:** 273–280.

Shibata D (1994). Extraction of DNA from paraffin-embedded tissue for analysis by polymerase chain reaction: new tricks from an old friend. *Hum Pathol* **25:** 561–563.

Singh N, Carrigan DR (1996). Human herpesvirus-6 in transplantation: an emerging pathogen. *Ann Intern Med* **124:** 1065–1071.

Suga S, Yoshikawa T, Asano Y *et al* (1992). IgM neutralizing antibody responses to human herpesvirus 6 in patients with exanthem subitum or organ transplantation. *Microbiol Immunol* **36:** 495–506.

Sullivan KM (1986). Acute and chronic graft-versus-host disease in man. *Int J Cell Cloning* **4** (Suppl. 1)**:** 42–93.

Wang FZ, Dahl H, Linde A, Brytting M, Ehrnst A, Ljungman P (1996). Lymphotropic herpesviruses in allogeneic bone marrow transplantation. *Blood* **88:** 3615–3620.

Wilborn F, Brinkmann V, Schimidt Ca Neipel F, Gelderblom H, Siegert W (1994). Herpesvirus type 6 in patients undergoing bone marrow transplantation: serologic features and detection by polymerase chain reaction. *Blood* **83:** 3052–3058.

Yadav M, Arivananthan M, Chandrashekran A, Tan BS, Hashim BY (1997). Human herpesvirus-6 (HHV-6) DNA and virus-encoded antigen in oral lesions. *J Oral Pathol Med* **26:** 393–401.

Yoshikawa T (2004). Human herpesvirus 6 infection in hematopoietic stem cell transplant patients. *Br J Haematol* **124:** 421–432.

Yoshikawa T, Suga A, Asano Y *et al* (1991). Human herpesvirus 6 infection in bone marrow transplantation. *Blood* **78:** 1381–1384.

Zerr DM, Gooley Ta, Yeung L *et al* (2001). Human herpesvirus 6 reactivation and encephalitis in allogeneic bone marrow transplant recipients. *Clin Infect Dis* **33:** 763–771.