

CYTOMEGALOVIRUS AND OTHER HERPESVIRUSES INFECTIONS IN HEART AND BONE MARROW TRANSPLANT RECIPIENTS

Adriana WEINBERG (1,2), Lucy S. VILAS BOAS (1), Olavo FEHER (3), Tania M. V. STRABELLI (4), Maria Cristina D. S. FINK (1), Frederico L. DULLEY (3), David E. UIP (2, 4), Giovanni BELLOTTI (5), Dalton A. F. CHAMONE (3), Vicente AMATO NETO (2) & Cláudio S. PANNUTI (1).

SUMMARY

From January 1988 to January 1989 all the heart transplant and bone marrow recipients at the Instituto do Coração of the Hospital das Clínicas of the University of São Paulo Medical School were studied for the incidence and morbidity associated with herpesviruses infections after transplantation. Five bone marrow and 5 heart transplant recipients were followed for a mean of 4.2 months post-transplantation. All the patients were seropositive for cytomegalovirus (CMV) before admission and 80% experienced one or more recurrences during the observation period. Of the 12 episodes of CMV infection, that were identified in this study, 83% were accompanied by clinical or laboratory abnormalities. However, there was only one case of severe disease. The overall incidence of infection for herpes simplex (HSV) was 50%. Although most of HSV reactivations were oral or genital, one case of HSV hepatitis occurred. One of the 6 episodes of HSV infections that were treated with acyclovir showed an unsatisfactory response and was successfully managed with ganciclovir. All the individuals had anti-varicella zoster virus antibodies, but none of them developed infection. The study emphasizes the importance of active diagnostic surveillance of herpesvirus infections in transplant patients. Both CMV and HSV reactivations showed high incidence and important morbidity and thus, deserve prophylactic therapy.

KEY WORDS: Cytomegalovirus infection; Herpesviruses infection; Immunocompromised host; Transplantation.

INTRODUCTION

Herpesviruses are notable for their ubiquity, latency and tendency to reactivate in the immunosuppressed host. Of all the herpesviruses, cytomegalovirus (CMV) is the agent most frequen-

This work was supported by the E. J. Zerbini Foundation and CNPq, grant no. 408741-88.

(1) Virology laboratory of the Instituto de Medicina Tropical, São Paulo, HC-LIM-52.

(2) Division of Infectious Diseases of the Hospital das Clínicas of the University of São Paulo

(3) Division of Hematology, HC-FMUSP.

(4) Infection Control Committee of the Instituto do Coração of the HC-FMUSP (Incor).

(5) Clinical Division of the Incor.

Address for correspondence: Dr. Claudio Sérgio Pannuti, Instituto de Medicina Tropical de São Paulo, Laboratório de Virologia, Av. Dr. Enéas de Carvalho Aguiar, 470, CEP 05403 São Paulo, SP, Brasil.

tly associated with serious disease and death^{3, 6, 12, 13, 17, 23}. A number of factors contribute to the severity of cytomegalovirus infection in transplant recipients. Primary infections as opposed to reactivations with the virus, the use of immunosuppressive regimens containing anti-lymphocyte antibodies and high doses of corticosteroids appear to be associated with more serious illness^{8, 19, 20}. In addition, the type of transplant operation is an important determinant of the morbidity due to CMV. For instance, heart transplant recipients show the highest rates of cytomegalovirus reactivation, close to 100%, and bone marrow recipients have more often pneumonia, the most severe complication of CMV activity.

Infections caused by herpes simplex virus (HSV) in many immunocompromised hosts are unusually severe, slow to heal and associated with prolonged viral shedding³². Recurrent HSV episodes in these patients are often associated with substantial pain and progressive ulceration. Furthermore, spread of infection to contiguous or distant mucocutaneous sites or visceral organs may complicate uncontrolled local lesions. Zoster in the transplant patients is also more likely to be severe and prolonged, to lead to scarring and disseminate²⁷.

Reports on the incidence and morbidity of herpesviruses infections in transplant patients from several North American centers are available. Comparable information in our country were not collected until recently. This study reports the first results of a prospective survey of viral infections in heart and bone marrow recipients at the Instituto do Coração of the Hospital das Clínicas of the University of São Paulo Medical School. Data have been collected to establish the frequency and severity of CMV, HSV and varicella-zoster virus (VZV) infections, to identify risk factors and determine prophylactic policies.

PATIENTS AND METHODS

Between January 1988 and January 1989 all the patients admitted for either cardiac or bone marrow transplantation at the Instituto do Coração of the Hospital das Clínicas of the Univer-

sity of São Paulo Medical School (Incor) were studied prospectively for viral infection. The subjects were daily evaluated and the following clinical data were weekly recorded on the viral study protocol: fever, respiratory insufficiency, diarrhea, gastro-intestinal bleeding, nausea and vomiting, jaundice, liver, spleen, or lymphnodes enlargement and presence of mucocutaneous lesions suggestive of viral infection. The subjects were also given routine chest radiograms, complete blood cell counts, serum liver enzymes, bilirubin and amylase. Other diagnostic tests such as bone marrow examination, endoscopy, biopsy, ophthalmologic evaluation were performed as clinically indicated. Immunosuppressive medication, blood transfusions rejection episodes, graft-versus-host disease and bacterial and fungal infections were also recorded.

Viral Diagnosis

Specimens for virus isolation and sera for antibody determination were collected weekly during hospital admission and at clinic visits thereafter.

Viral isolation. Urine, blood and oral swabs were routinely inoculated onto human foreskin fibroblasts, Vero and Hela monolayers. Genital swabs and biopsy specimens were also studied when clinically indicated. The inoculated human foreskin fibroblasts were observed for 4 weeks and the Vero and Hela cultures for 2 weeks. Viruses were identified by their typical cytopathic effect and their presence was confirmed, when needed, by electron microscopy, direct immunofluorescence (DFA) using anti-CMV monoclonal antibodies (Du Pont — NEN Research Products, Boston, Ma) and dotblot with guinea-pig anti-HSV hyperimmune serum^{11, 31}.

Antibody measurement. Antibodies were determined in the pretransplant sera and weekly thereafter. Anti-cytomegalovirus, herpes simplex and varicella zoster IgG was measured by enzyme linked immunosorbent assay (ELISA), using antigens prepared in our laboratory, as previously described³⁰. IgM antibodies anti-CMV and HSV and IgG anti-CMV were identified by indirect immunofluorescence assay (IFA).

The technique of the IFA has already been published¹⁸.

Active viral infection was indicated by viral isolation, presence of IgM antibodies, of fourfold rise of the IgG titers.

RESULTS

Characteristics of the Study Population

Between January 1988 and January 1989, 10 patients were enrolled in this study. Five individuals underwent heart transplantation and the remaining 5 were allogeneic bone marrow recipients. There were 6 male patients and 4 females. Their ages ranged from 3 to 45 years, with a mean of 26.8 ± 12.3 years. The bone marrow transplant subjects tended to be younger (mean of 18.2 and range of 3 to 31 years) as compared to the heart recipients (mean of 35 and range of 26 to 45 years). The patients were studied for an average of 4.2 months post-transplantation, ranging from 1 to 10 months. Post-transplant management of heart recipients included the use of cyclosporin, prednisone and azathioprine. Methylprednisolone was employed for acute rejection. Bone-marrow transplant patients received cyclosporin and methotrexate. All the heart transplants had one or more successfully treated episodes of rejection while on protocol. Anti-lymphocyte globulin was not available during this study. Two bone marrow patients were retransplanted during the study. Acute graft-versus-host disease was found in 2 patients. Antiviral prophylactic drugs were not used routinely, although some bone marrow transplant individuals randomly received prophylactic acyclovir for brief periods of time. Two bone marrow recipients died during the study.

Cytomegalovirus

All the patients were seropositive with respect to CMV before transplantation. During the virologic survey, 8 individuals, 4 heart and 4 bone marrow recipients, experienced one or more episodes of active cytomegalovirus infection. The virus was identified only in the urine in 2 patients, only in the oral secretions in 3 cases and in both urine and oral secretions in another 3

individuals. On one occasion, CMV was detected in the blood and small bowel biopsy specimen. In these cases the viral shedding was present for a mean of 23 ± 15 days. Four additional episodes of CMV reactivation were identified by specific anti-CMV IgM production and fourfold rise of anti-CMV IgG antibodies solely (table 1).

The 12 episodes of CMV reactivation occurred 88.6 ± 67.8 days post-transplantation. There was no relationship between CMV replication and transfusion, rejection or graft-versus-host disease. All the heart transplant patients and 2 bone marrow recipients showed rejection during the study. Among them, 4 heart transplant individuals and 1 bone marrow transplant subject had CMV reactivation. All the heart recipients had blood transfusions 4 to 8 weeks before the episode of active cytomegalovirus infection and all the bone marrow transplant patients were also submitted to blood and platelet transfusions. Granulocytes were not administered during this study. Two bone marrow recipients underwent acute graft-versus-host disease. One of them also showed concurrent cytomegalovirus active replication.

Of the 12 episodes of CMV reactivation/reinfection, 2 were completely asymptomatic. The remaining 10 were associated with clinical manifestations that might have been caused by cytomegalovirus. The most frequently observed abnormalities concurrent with CMV infection were liver function test disorders (5 cases). In one of the patients there were additional signs and symptoms of hepatitis, including jaundice. None of the 5 subjects had liver biopsies performed. Fever was present in 3 out of the 12 CMV reactivations. However, one of the patients had a concurrent fungal infection. Overall, there were hematologic disorders possibly due to CMV activity in 2 heart and 2 marrow recipients. One of the heart transplant subjects had a bone marrow biopsy performed which showed diffuse hypocellularity. A bone marrow recipient had severe diarrhea and intestinal bleeding while CMV was isolated from a small bowel biopsy and from the blood. This patient, however, was also undergoing intestinal graft-versus-host disease which might have contributed to the clinical manifestations. There were 6 episodes of fungal or bacte-

WEINBERG, A.; VILAS BOAS, L. S.; FEHER, O.; STRABELLI, T. M. V.; FINK, M. C. D. S.; DULLEY, F. L.; UIP, D. E.; BELLOTTI, G.; CHAMONE, D. A. F.; AMATO NETO, V. & PANNUTI, C. S. — Cytomegalovirus and other herpesviruses infections in heart and bone marrow transplant recipients. *Rev. Inst. Med. trop. S. Paulo*, 32(5): 338-345, 1990.

TABLE 1
Clinical and laboratory manifestations associated with active cytomegalovirus infection in transplant recipients.

Event no.	Age	T ^a Type	Time post-T	Drugs	Diagnosis	Manifestation	Therapy	Outcome
1	32	HT	22	Cs, P ^b Aza	oral swab. IgM	>AP ^c	none	recovered
2	45	HT	33	Cs, P Aza	urine, IgM oral swab,	> SGPT, > SGOT, > AP	none	recovered
3	37	HT	54	Cs, P Aza	IgM	fever, leucopenia ^e	none	recovered
4	26	HT	41	Cs, P Aza	urine, > IgG ^d , IgM	fever, thrombocytopenia ^e	none	recovered
5	3	BMT	4	Cs	oral swab	none	none	recovered
6	3	BMT	89	Cs	> Ig	> AP	none	recovered
7	3	BMT	131	Cs	> IgG	> AP	none	recovered
8	17	BMT	31	Cs	> IgG	none	none	recovered
9	31	BMT	82	Cs	oral swab, urine, IgM	> SGPT, > SGOT, > AP, leukopenia	none	recovered
10	16	BMT	117	CS	IgM	pancytopenia ^e	none	recovered
11	16	BMT	210	Cs, S	urine	none	none	recovered
12	16	BMT	250	Cs, S	blood, small bowel	fever, diarrhea, intestinal bleeding	ganciclovir	died

a) T = transplant; HT = heart transplant; BMT = bone marrow transplant.

b) Cs = cyclosporine A; P = prednisone; Aza = azathioprine; S = methylprednisolone;

c) >AP = increased alkaline phosphatase; > SGPT = increased serum aspartate aminotransferase; > SGOT = increased serum alanine aminotransferase.

d) > IgG = fourfold or higher increase of anti-CMV IgG serum titer.

e) leukopenia indicates leukocytes < 3000 cells/ml; thrombocytopenia indicates platelets < 100,000/ml; important anemia was considered when Hb < 10 g/ml.

rial infection associated with CMV active replication. In contrast, only 3 patients had non-viral super-infection in the absence of cytomegalovirus reactivation.

Only episode no. 12 of cytomegalovirus infection was considered for specific treatment with human gammaglobulin and ganciclovir. Despite the therapeutic efforts, the patient died with severe graft-versus-host disease and unsuspected fungal infection. At necropsy, CMV inclusions were still present in the small bowel.

Herpes simplex

Nine out of the 10 patients enrolled in this study had antibodies anti-HSV prior to transplantation. In 5 of these individuals (2 heart and 3 marrow recipients) herpes simplex reactivated on 6 occasions, at 113 ± 97 days post-transplantation. The HSV infections were identified

by isolating the virus from oral secretions, from perineal swabs or blood (table 2). Furthermore, 2 bone marrow transplant recipients also produced anti-herpes IgM in response to the viral replication. All the episodes of HSV infection were accompanied by clinical symptoms. Four patients had local reactivations: 3 with oral-labial manifestations and one case with genital lesions. One patient showed clinical and laboratory hepatitis associated with viremia and asymptomatic oral HSV excretion. A liver biopsy was not obtained, but the patient improved on intravenous acyclovir. The remaining HSV reactivation episode represented disseminated disease, since the patient displayed both oral and genital lesions and the virus was identified at these sites and in the urine too.

All the patients received acyclovir after the diagnosis of active herpes infection was established. In all but one case there was a satisfactory

WEINBERG, A.; VILAS BOAS, L. S.; FEHER, O.; STRABELLI, T. M. V.; FINK, M. C. D. S.; DULLEY, F. L.; UIP, D. E.; BELLOTTI, G.; CHAMONE, D. A. F.; AMATO NETO, V. & PANNUTI, C. S. — Cytomegalovirus and other herpesviruses infections in heart and bone marrow transplant recipients. *Rev. Inst. Med. trop. S. Paulo*, 32(5): 338-345, 1990.

TABLE 2
Clinical and laboratory manifestations of Herpes simplex infections in transplant recipients.

Event no.	Age	Type	Time post-T	Drugs	Diagnosis	Manifestation	Therapy	Outcome
1	45	HT	33	Cs, P Aza	oral swab,	oral-labial	acyclovir	recovered
2	37	HT	27	Cs, P Aza	peri-anal swab	genital	acyclovir	recovered
3	3	BMT	101	Cs	oral swab	oral-labial	acyclovir	recovered
4	31	BMT	54	Cs	oral swab, blood, IgM	hepatitis	acyclovir	recovered
5	16	BMT	203	Cs, S	oral swab	oral-labial	acyclovir	recovered
6	16	BMT	260	Cs, S	oral swab, urine, IgM cervical swab	oral-labial, genital	acyclovir ganciclovir	persisted recovered

clinical response and the viral shedding was no longer present after 7 days of therapy. One bone marrow transplant recipient presented with lesions that did not improve with acyclovir and the virus excretion continued after 2 weeks of therapy. This individual was eventually treated with ganciclovir, for a concurrent CMV infection, and HSV was no longer detected at the following weekly control.

Two patients, a bone marrow and a heart recipient showed 3 episodes of simultaneous HSV and CMV infections, determined by viral culture. The agents were specifically identified by dot-blot and direct immunofluorescence, respectively. In one case, the patient had genital herpes and hepatitis. The latter was ascribed to the cytomegalovirus, because it was present before HSV had been isolated and, in contrast to the genital lesions, it did not respond to acyclovir. Another individual had small bowel CMV infection associated with disseminated HSV disease on one occasion and another episode of oral-labial herpes and decrease of all blood cell series which might have been due to CMV infection.

Varicella-zoster virus

Although all the patients presented with anti-VZV IgG antibodies measured by ELISA, prior to transplantation, none of them had zoster during the study.

DISCUSSION

This prospective virologic study emphasizes the importance of cytomegalovirus infections in transplant patients. The rate of infection that we found, 80% in both heart and marrow recipients, is in accordance with other studies where the frequency of CMV infection varied between 50 and 100%^{6, 20}. Clinical and laboratory abnormalities were present in 83% of the episodes (10 out of 12) which suggests that symptomatic disease might be more common than it was previously reported (30%)^{6, 19, 23}. Liver function tests abnormalities were the most frequently observed disorders followed by fever, leukopenia and thrombocytopenia. There were no cases of CMV pneumonitis, the most feared complication of cytomegalovirus infection, that carries an 85% mortality rate and accounts for 50% of the deaths in bone marrow transplant subjects. However, there was one episode of severe CMV gastro-intestinal disease performing an 8% rate of serious illness. Cytomegalovirus infection has been shown to predispose to bacterial and fungal superinfection in transplant patients^{9, 21, 23}. In this study, CMV replication was associated with 6 of the 9 episodes of nonviral infection.

The risk of developing a CMV infection post-transplantation was shown to depend on several factors: recipient seropositivity, use of high dose corticosteroids and anti-lymphocyte globulin for reactivations; donor seropositivity, number of

blood transfusions and particularly granulocytes administration for primary infection^{8, 19, 20, 25}. More recently, it has been demonstrated that seropositive transplant patients may be reinfected with a different CMV strain after transplantation¹⁰. In the setting of reinfection the hosts might develop overt disease more frequently and of increased severity when compared with reactivation infection subjects. Reinfections were associated with elevated number of blood transfusions and donor seropositivity. In this study, the donor status was not always available and the incidence of CMV infection did not vary according to the blood transfusions. Furthermore, we did not observe any clear positive or negative correlation between cytomegalovirus replication and rejection or graft-versus-host disease, respectively, as it has been suggested^{2, 16}. However, in the heart transplant patients all the CMV infections occurred simultaneously with graft rejections. The same observation was previously reported in renal transplant patients¹³. Although this parallel in onsets may have reflected only a coincidental similarity of host-versus-graft disease and the incubation period of CMV infection, there might be a more fundamental relationship between the two events.

Important progress has been recently achieved in the treatment of cytomegalovirus infections. Several studies demonstrated good results with the use of ganciclovir in immunocompromised hosts with severe CMV disease added of hyperimmune gammaglobulin in cases of CMV pneumonia^{7, 22}. Because ganciclovir therapy carries a significant risk of leukopenia, azospermia and minor renal and hepatic toxicity, treatment has been recommended only in cases of life or vision threatening infections. In this study only one patient fulfilled the aforementioned criteria. Foscarnet is a less toxic drug that has shown, in preliminary communications, comparable efficacy with ganciclovir²⁹. If confirmed, this finding might determine a broader indication for specific anti-CMV therapy. Furthermore, severe infections with cytomegalovirus could be prevented in transplant patients who received acyclovir prophylaxis or anti-CMV hyperimmune globulin^{1, 4, 15, 26, 33}. Ganciclovir is also being evaluated for prophylaxis in heart and bone marrow recipients.

As reported in other organ allograft recipients, HSV infections were quite common (50%) and mostly due to reactivation of latent virus. Mucocutaneous non-severe infections were found in 40% of the patients and were easily managed with antiviral therapy. A similar good therapeutic response was observed in a patient with presumed herpetic hepatitis. However, a bone marrow recipient who had previously received therapeutic acyclovir, developed resistant HSV disease, that responded only to ganciclovir. The emergence of acyclovir resistant strains of pathogenic herpes simplex has not been a problem in the normal host, even after long term prophylaxis and it has rarely been described in immunocompromised patients^{5, 24}. Because of the ubiquity of HSV infections and the high reactivation rate in transplant recipients most bone marrow transplant centers have routinely used prophylactic acyclovir during the first 4 months post-transplantation.

VZV infections were not detected in this study. This was not unexpected, since the rate of VZV reactivation in transplant recipients is below 10%^{6, 27}.

It is interesting to note that concurrent herpes simplex and cytomegalovirus infections were observed on 3 occasions, in 2 patients. This finding argues against viral inhibition between CMV and HSV in immunocompromised hosts and underscores the need of accurate diagnostic methods.

The study emphasizes the importance of active diagnostic surveillance of herpesvirus infections in transplant patients. Both CMV and HSV reactivations were quite common in the heart and marrow transplant recipients and carried important morbidity. We conclude that these patients deserve prophylactic therapy.

RESUMO

Infecções causadas por citomegalovírus e outros vírus do grupo herpes em transplantados cardíacos e de medula óssea.

De janeiro de 1988 a janeiro de 1989 todos os pacientes submetidos a transplante de coração ou de medula óssea no Instituto do Coração

do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo foram estudados quanto à incidência e morbidade das infecções pós-transplante causadas por vírus do grupo herpes. Cinco recipientes de medula óssea e 5 transplantados cardíacos foram observados por um período médio de 4.2 meses após o transplante. Todos os pacientes tinham sorologia positiva para citomegalovírus (CMV) antes do transplante e 80% desenvolveram uma ou mais recorrências durante o período de observação. Dos 12 episódios de infecção por CMV detectados neste estudo, 83% foram acompanhados por alterações clínicas ou laboratoriais. Apenas um caso apresentou doença grave. A incidência de infecções causadas por vírus Herpes simplex (HSV), foi de 50%. Apesar da maioria das reativações do HSV fossem representadas por lesões orais ou genitais, houve também um caso de hepatite por HSV. Um dos 6 episódios de infecção, por HSV que foram tratados com aciclovir não respondeu ao tratamento. Posteriormente, o paciente se beneficiou com o uso de ganciclovir. Todos os indivíduos apresentavam antes do transplante anticorpos anti-vírus da varicela zoster. Porém, não houve nenhum caso de reativação. Este estudo realça a importância do controle diagnóstico ativo das infecções por herpesvírus em pacientes transplantados. Tanto as infecções causadas por CMV como por HSV mostraram alta incidência e grande morbidade indicando a necessidade de quimioprofilaxia.

REFERENCES

1. BALFOUR, H. H.; CHACE, B. A.; STAPLETON, J. T.; SIMMONS, R. J. & FRYD, D. S. — A randomized, placebo controlled trial of oral acyclovir for the prevention of cytomegalovirus disease in recipients of renal allografts. *New Engl. J. Med.*, 320: 1381-1387, 1989.
2. BOSTROM, L.; RINGDEN, O.; SUNDBERG, B.; LINDE, A.; TOLLEMAR, J. & NILSSON, B. — Pretransplant herpesvirus serology and acute graft-versus-host disease. *Transplantation*, 46: 548-552, 1988.
3. BREINING, M. K.; ZITELLI, B.; STARZL, T. E. & HO, M. — Epstein-Barr virus, cytomegalovirus, and other viral infections in children after liver transplantation. *J. infect. Dis.*, 156: 273-279, 1987.
4. CONDIE, R. M. & O'REILLY, R. J. — Prevention of cytomegalovirus infection by prophylaxis with an intravenous, hyperimmune, native, unmodified cytomegalovirus globulin. Randomized trial in bone marrow transplant recipients. *Amer. J. Med.*, 76: 134-141, 1984.
5. DORSKY, D. Y. & CRUMPACKER, C. S. — Drugs five years later: acyclovir. *Ann. intern. Med.*, 107: 859-874, 1987.
6. DUMMER, J. S.; HARDY, A.; POORSATTAR, A. & HO, M. — Early infections in kidney, heart and liver transplant recipients on cyclosporine. *Transplantation*, 36: 259-267, 1983.
7. EMMANUEL, D.; CUNNINGHAM, I.; JULES-ELYSEE, K.; BROCHSTEIN, J. A.; KERNAN, N. A.; LAVER, J.; STOVER, D.; WHITE, D. A.; FELS, A.; POLSKY, B.; CASTRO-MALASPINA, H.; PEPPARD, J. R.; BARTUS, P.; HAMMERLING, U. & O'REILLY, R. J. — Cytomegalovirus pneumonia after bone marrow transplantation successfully treated with the combination of ganciclovir and high-dose intravenous immune globulin. *Ann. intern. Med.*, 109: 777-782, 1988.
8. GORENSEK, M. J.; STEWART, R. W.; KEYS, T. F.; MCHENRY, M. C. & GOORMASTIC, M. — A multivariate analysis of the risk of cytomegalovirus infection in heart transplant recipients. *J. infect. Dis.*, 157: 515-522, 1988.
9. GORENSEK, M. J.; STEWART, R. W.; KEYS, T. F.; MCHENRY, M. C.; BABIAK, T. & GOORMASTIC, M. — Symptomatic cytomegalovirus infection as a significant risk factor for major infections after cardiac transplantation. *J. infect. Dis.*, 158: 884-887, 1987.
10. GRUNDY, J. E.; SUPER, M.; SWENY, P.; MOORHEAD, J.; LUI, S. F.; BERRY, N. J.; FERNANDO, O. N. & GRIF-FITHS, P. D. — Symptomatic cytomegalovirus infection in seropositive kidney recipients: reinfection with donor virus rather than reactivation of recipient virus. *Lancet*, 2: 132-135, 1988.
11. HAWKES, R.; NIDAY, E. & GORDON, J. — A dot-immunobinding assay for monoclonal and other antibodies. *Ann. Biochem.*, 119: 142-147, 1982.
12. KUSNE, S.; DUMMER, J. S.; SINGH, N.; IWATSUKI, S.; MAKOWKA, L.; ESQUIVEL, C.; TZIKIS, A. G.; STARZL, T. E. & HO, M. — Infections after liver transplantation. An analysis of 101 consecutive cases. *Medicine*, 67: 132-143, 1988.
13. MARKER, S. C.; HOWARD, R. J.; SIMMONS, R. L.; KALLIS, J. M.; CONNELLY, D. P.; NAJARIAN, J. S. & BALFOUR Jr., H. H. — Cytomegalovirus infection: a quantitative prospective study of three hundred twenty consecutive renal transplants. *Surgery*, 89: 660-671, 1981.
14. MEYERS, J. D.; LESZYNSKI, J.; ZAIA, J. A.; FLOURNOY, N.; NEWTON, B.; SNYDMAN, D. R.; WRIGHT, G. G.; LEVIN, M. J. & THOMAS, E. D. — Prevention of cytomegalovirus infection by cytomegalovirus immune globulin after marrow transplantation. *Ann. intern. Med.*, 98: 442-446, 1983.
15. MEYERS, J. D.; REED, E. C.; SHEPP, D. H.; THORNTON, M.; DANGLIKER, P. S.; VICARY, C. A.; FLOURNOY, N.; KIRK, L. E.; KERSEY, J. H.; THOMAS, E. D. & BALFOUR, H. H. — Acyclovir for prevention of cytomegalovirus infection and disease after allogeneic marrow transplantation. *New Engl. J. Med.*, 318: 70-75, 1988.

WEINBERG, A.; VILAS BOAS, L. S.; FEHER, O.; STRABELLI, T. M. V.; FINK, M. C. D. S.; DULLEY, F. L.; UIP, D. E.; BELLOTTI, G.; CHAMONE, D. A. F.; AMATO NETO, V. & PANNUTI, C. S. — Cytomegalovirus and other herpesviruses infections in heart and bone marrow transplant recipients. *Rev. Inst. Med. trop. S. Paulo*, 32(5): 338-345, 1990.

16. O'GRADY, J. G.; SUTHERLAND, S.; HARVEY, F.; CAYNE, R. Y.; ALEXANDER, G. J.; DONALDSON, P. T.; PORTMANN, B. & WILLIAMS, R. — Cytomegalovirus infections and donor/recipient HLA antigens: interdependent co-factors in pathogenesis of vanishing bileduct syndrome after transplantation. *Lancet*, 2: 302-305, 1988.
17. PANNUTI, C. S.; GINGRICH, R. D.; PFALLER, M. A. & WENZEL, R. P. — Nosocomial pneumonia in adult patients undergoing bone marrow transplantation: a nine year study (Submitted for publication, 1989).
18. PANNUTI, C. S.; VILAS BOAS, L. S.; AMATO NETO, V.; ANGELO, M. J. O. & SABBAGA, E. — Detecção de anticorpos IgM nas infecções primárias e secundárias pelo citomegalovirus em pacientes submetidos a transplante renal. *Rev. Inst. Med. trop. S. Paulo*, 29: 317-322, 1987.
19. PETERSON, P. K.; BALFOUR, H. H.; MARKER, S. C.; FRYD, D. S.; HOWARD, R. J. & SIMMONS, R. L. — Cytomegalovirus disease in renal allograft recipients: a prospective study of the clinical features, risk factors and impact on renal transplantation. *Medicine*, 59: 283-300, 1980.
20. PREIKSAITIS, J. K.; ROSNO, S.; GRUMET, C. & MERIGAN, T. C. — Infection due to herpesviruses in cardiac transplant recipients: role of donor heart and immunosuppressive therapy. *J. infect. Dis.*, 147: 974-981, 1983.
21. RAND, K. H.; POLLARD, R. B. & MERIGAN, T. C. — Increased pulmonary superinfection in cardiac transplant patients undergoing primary cytomegalovirus infection. *New Engl. J. Med.*, 288: 951-953, 1978.
22. REED, E. C.; BOWDEN, R. A.; DANDLIKER, P. S.; LILLEBY, K. E. & MEYERS, J. D. — Treatment of cytomegalovirus pneumonia with ganciclovir and intravenous cytomegalovirus immunoglobulin in patients with bone marrow transplants. *Ann. intern. Med.*, 109: 783-788, 1988.
23. RUBIN, R. H.; COSIMI, A. B.; TOLKOFF-RUBIN, N. E.; RUSSELL, R. S. & HIRSCH, M. — Infectious disease syndromes attributable to cytomegalovirus and their significance among renal transplant recipients. *Transplantation*, 24: 458-464, 1977.
24. SHEPP, D. H.; NEWTON, B. A.; DANDLIKER, P. S.; FLOURNOY, N. & MEYERS, J. D. — Oral acyclovir therapy for mucocutaneous herpes simplex virus infections in immunocompromised marrow transplant recipients. *Ann. intern. Med.*, 102: 783-785, 1985.
25. SINGH, N.; DUMMER, J. S.; KUSNE, S.; BREINIG, M. K.; ARMSTRONG, J. A.; MAKOWKA, L.; STARZL, T. E. & HO, M. — Infections with cytomegalovirus and other herpesviruses in 121 liver transplant recipients: transmission by donated organ and the effect of OKT3 antibodies. *J. infect. Dis.*, 158: 124-131, 1988.
26. SNYDMAN, R. D.; WERNER, B. G.; HEINZE-LACEY, B.; BERARDI, V. P.; TILNEY, N. L.; KIRKMAN, R. L.; MILFORD, E. L.; CHO, S. I.; BUSH, H. L.; LEVEY, A. S.; STROM, T. B.; CARPENTER, C. B.; LEVEY, R. H.; HARMON, W. E.; ZIMMERMAN, C. E.; SHAPIRO, M. E.; STEINMAN, T.; LOGERFO, F.; IDELSON, B.; SCHROTER, G. P. J.; LEVIN, M. J.; MCIVER, J.; LESZYNSKI, J. & GRADY, G. F. — Use of cytomegalovirus immunoglobulin to prevent cytomegalovirus disease in renal transplant recipients. *New Engl. J. Med.*, 317: 1049-1054, 1987.
27. STRAUS, S. E.; OSTROVE, J. M.; INCHAUSPE, G.; FELSER, J. M.; ALISON FREIFELD, A.; CROEN, K. D. & SAWYER, M. H. — Varicella-zoster virus infections biology, natural history, treatment and prevention. *Ann. intern. Med.*, 108: 221-237, 1988.
28. WADE, J. C.; NEWTON, B.; FLOURNOY, N. & MEYERS, J. D. — Oral acyclovir for prevention of herpes simplex reactivation after marrow transplant. *Ann. intern. Med.*, 100: 823-828, 1984.
29. WALMSLEY, S. L.; CHEW, E.; READ, S. E.; VELLEND, H.; SALIT, I.; RACHLIS, A. & FANNING, M. M. — Treatment of cytomegalovirus retinitis with trisodium phosphonoformate hexahydrate (Foscarnet). *J. infect. Dis.*, 157: 569-572, 1988.
30. WEINBERG, A.; BASHAM, T. Y. & MERIGAN, T. C. — Regulation of the guinea pig immune functions by interleukin-2: critical role of natural killer activity in acute HSV-2 genital infection. *J. Immunol.*, 137: 3310-3316, 1986.
31. WEINBERG, A.; RASMUSSEN, L. & MERIGAN, T. C. — Acute genital infection in guinea pigs: effects of recombinant interleukin-2 on herpes simplex virus type 2. *J. infect. Dis.*, 154: 134-138, 1986.
32. WHITLEY, R. J.; LEVIN, M. J.; BARTON, N.; HERSHEY, B. J.; DAVIS, G.; KEENEY, R. E.; WHELCHER, J.; DIETHELM, A. G.; KARTUS, P. & SOONG, S. — Infections caused by herpes simplex virus in the immunocompromised host: natural history and topical acyclovir therapy. *J. infect. Dis.*, 150: 323-329, 1984.
33. WINSTON, D. J.; HO, G. H.; LIN, C.; BARTONI, K.; BUDINGER, M. D.; GALE, P. R. & CHAMPLIN, R. E. — Intravenous immune globulin for prevention of cytomegalovirus infection and interstitial pneumonia after bone marrow transplantation. *Ann. intern. Med.*, 106: 12-18, 1987.

Recebido para publicação em 23/1/1990