

TRANSMISSION OF HUMAN HERPESVIRUS 8 INFECTION FROM RENAL-TRANSPLANT DONORS TO RECIPIENTS

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ABSTRACT

Background Human herpesvirus 8 (HHV-8) has been detected in all forms of Kaposi's sarcoma, including transplantation-associated Kaposi's sarcoma. To investigate the possibility of transmission of HHV-8 through allografts, we measured the seroprevalence of HHV-8 before and after renal transplantation.

Methods Using an enzyme-linked immunosorbent assay with the recombinant HHV-8 protein orf 65.2, we analyzed serum samples from 220 renal-transplant recipients for the presence of antibodies to HHV-8 on the day of transplantation and one year later. Positive results were confirmed by an indirect immunofluorescence assay that detects antibodies to latent antigen and by Western blotting. Follow-up lasted at least four years.

Results The seroprevalence of HHV-8 in graft recipients increased from 6.4 percent on the day of transplantation to 17.7 percent one year after transplantation. Seroconversion occurred within the first year after transplantation in 25 patients, and Kaposi's sarcoma developed in 2 of them within 26 months after transplantation. Sequential serum samples were obtained from 10 of the patients with seroconversion, and in 8 of these patients, IgM antibodies to HHV-8 appeared within three months after transplantation. In the case of six patients who seroconverted, serum samples from the donors were available, and five (83 percent) tested positive for HHV-8. In a control group of eight patients who were seronegative at the time of transplantation and who received allografts from HHV-8-negative donors, none seroconverted within the year after transplantation.

Conclusions HHV-8 is transmitted through renal allografts and is a risk factor for transplantation-associated Kaposi's sarcoma. (N Engl J Med 1998; 339:1358-63.)

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THE recently described human herpesvirus 8 (HHV-8, also known as Kaposi's sarcoma-associated herpesvirus) can be detected in all forms of Kaposi's sarcoma.¹⁻⁴ HHV-8 infection, identified by the presence of HHV-8 DNA sequences in peripheral-blood mononuclear cells or by the presence of serum antibodies to HHV-8, both precedes and predicts the onset of Kaposi's sarcoma in patients infected with the human immunodeficiency virus (HIV).⁵⁻⁷

After solid-organ transplantation, there is a marked increase in epithelial skin tumors, malignant lym-

phomas, and Kaposi's sarcoma.⁸ Transplantation-associated Kaposi's sarcoma occurs in 0.2 to 5 percent of renal-transplant recipients⁹⁻¹⁴; the incidence varies among racial and ethnic groups and among groups of patients treated with different immunosuppressive regimens.^{9,15} HHV-8 DNA has been detected in Kaposi's sarcoma lesions but not in other malignant tumors associated with renal transplantation.¹⁴

The route of HHV-8 transmission is not yet fully understood, although there is evidence that the virus can be transmitted sexually.¹⁶⁻¹⁹ Because Kaposi's sarcoma is associated with HHV-8 infection and the incidence of Kaposi's sarcoma is markedly increased after solid-organ transplantation, we assessed the seroprevalence of HHV-8 in renal-transplant recipients before and after transplantation.

METHODS

Patients

Between January 1988 and February 1994, a total of 347 patients received a renal allograft at the University Hospital of Basel in Basel, Switzerland. Serum samples obtained on the day of transplantation from 279 of these patients (mean age, 44.7 years; range, 7 to 71; 59 percent male patients and 41 percent female patients) were available for analysis. In the case of 220 of these patients, serum samples were obtained about 1 year after transplantation (133 samples obtained at 12 months and 87 samples obtained between 4 and 98 months; mean, 16.1). All patients were followed up for at least four years after transplantation. Age, sex, the underlying renal disease, the type of transplant (cadaveric or from a living donor), serologic status of the donor and the recipient with respect to cytomegalovirus (also termed human herpesvirus 5), the number of units of packed red cells received within the first year after transplantation, the immunosuppressive regimen used, graft survival, and the development of malignant conditions were recorded for all patients.

Expression of Recombinant HHV-8 Proteins and Enzyme-Linked Immunosorbent Assay

Recombinant orf 65.2 proteins, corresponding to amino acids 86 to 170 of the HHV-8 open reading frame (orf) 65, were expressed in M14 bacteria and purified by affinity chromatography on nickel-nitrilotriacetic acid resin (Qiagen, Basel, Switzerland).²⁰ An enzyme-linked immunosorbent assay (ELISA) was performed as described previously.¹⁸ Briefly, micro-ELISA plates (Greiner, Frickenhausen, Germany) were coated overnight with 100 μ l of purified orf 65.2 protein (2 μ g per milliliter) in 0.1 mol of sodium bicarbonate per liter (pH 8.5) at 4°C. The plates were washed

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with phosphate-buffered saline containing 0.1 percent polysorbate 20 (Tween 20) and saturated with 5 percent dried skim milk in phosphate-buffered saline containing 0.1 percent Tween 20 (as a blocking buffer). A 1:10 dilution was used for serum samples from the patients. For the detection of serum IgG antibodies, a peroxidase-conjugated goat antihuman IgG (Sigma, Buchs, Switzerland) was used (dilution, 1:2000); for the detection of serum IgM antibodies, a commercial human IgM antibody-detection kit (Enzygnost, Behringwerke, Marburg, Germany) was used as recommended by the manufacturers. To avoid false positive results, potential rheumatoid factors were removed beforehand.

The IgG or IgM cutoff value for each plate was determined by calculating the mean +5 SD of the optical-density values of serum samples from five blood donors. Two reactive serum samples from patients with Kaposi's sarcoma were used as positive controls for each plate. All serum samples were tested blindly in duplicate, and positive samples or samples with values close to the cutoff value were reanalyzed at least once to confirm the results. We have previously shown that the orf 65.2 antigen used in the ELISA does not cross-react with antibodies to other herpesviruses.¹⁸

All available serum samples from donor-recipient pairs as well as samples that were positive for HHV-8 on ELISA were tested as a means of confirmation by an indirect immunofluorescence assay that detects antibodies to a latency-associated nuclear antigen,¹⁶ as described elsewhere,¹⁸ and by Western blotting with recombinant orf 65.2 proteins.²⁰

Statistical Analysis

The seroprevalence of HHV-8 before and after transplantation was compared with the use of McNemar's test in a two-by-two table.

RESULTS

Seroprevalence of Antibodies to HHV-8 before and One Year after Transplantation

At the time of transplantation, serum samples from 14 of 220 patients (6.4 percent) had IgG antibodies to HHV-8 as measured by ELISA with orf 65.2 and by an immunofluorescence assay with latent antigen (Table 1). The results for four samples were borderline on ELISA but negative on the immunofluorescence assay and were therefore considered negative. One year after transplantation, 39 patients (17.7 percent) were seropositive. Seroconversion within the first year after transplantation occurred in 25 of 206 patients (12.1 percent) who were seronegative for HHV-8 before transplantation (P<0.001 by McNemar's test).

Seroprevalence of HHV-8 after Renal Transplantation

The seroconversion observed in 25 patients within the first year after transplantation strongly suggested the occurrence of donor-transmitted infection. To verify this hypothesis, we measured IgM antibody titers in 10 patients who seroconverted and for whom sequential serum samples were available (Fig. 1). Representative IgG and IgM antibody titers are shown for two patients in Figure 2.

In 8 of the 10 patients, IgM antibodies to HHV-8 appeared within three months after transplantation. A marked increase in the IgM antibody titer

TABLE 1. SEROPREVALENCE OF HHV-8 BEFORE AND ONE YEAR AFTER RENAL TRANSPLANTATION IN 220 PATIENTS.*

TIME OF MEASUREMENT	HHV-8 POSITIVE	HHV-8 NEGATIVE	TOTAL
	no. (%)		
Before transplantation	14 (6.4)	206	220
One year after transplantation	39 (17.7)	181	220
Seroconversion after transplantation	25 (12.1)†	181	206

*Seroprevalence was measured by enzyme-linked immunosorbent assay with orf 65.2 and confirmed by immunofluorescence assay with latent antigen.

†P<0.001 by McNemar's test for the comparison of seroprevalence before and after transplantation.

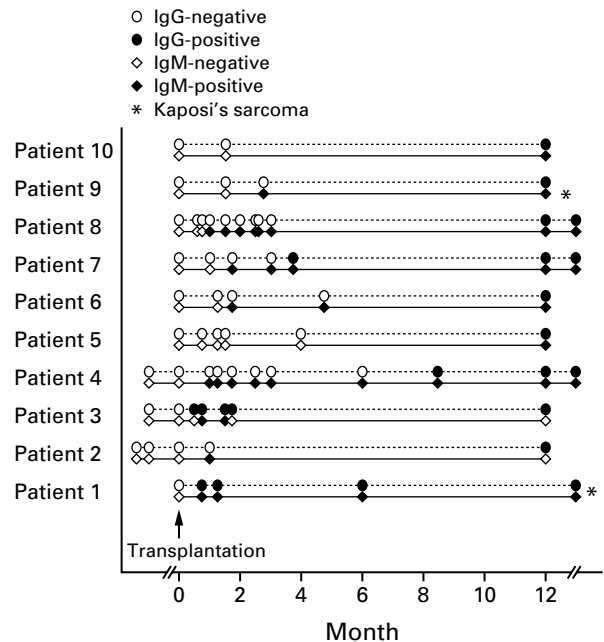


Figure 1. Time of Seroconversion in 10 Transplant Recipients. Values for IgG were considered positive if they were above the cutoff optical-density value of 0.6 (wavelength, 492 nm), and values for IgM were considered positive if they were above the cutoff value of 0.27 (wavelength, 450 nm), except in Patients 4 and 6, in whom positive IgM values were defined as those that were more than twice the pretransplantation level.

was found as early as two weeks after transplantation (Patient 3). In two of these patients, the IgM antibody titers fell to pretransplantation levels within the first year after surgery (Patients 2 and 3), whereas they stayed positive for at least one year after transplantation in the other six patients (Patients 1, 4, 6, 7, 8, and 9). Kaposi's sarcoma developed in two of these six patients (Patients 1 and 9). Four of the

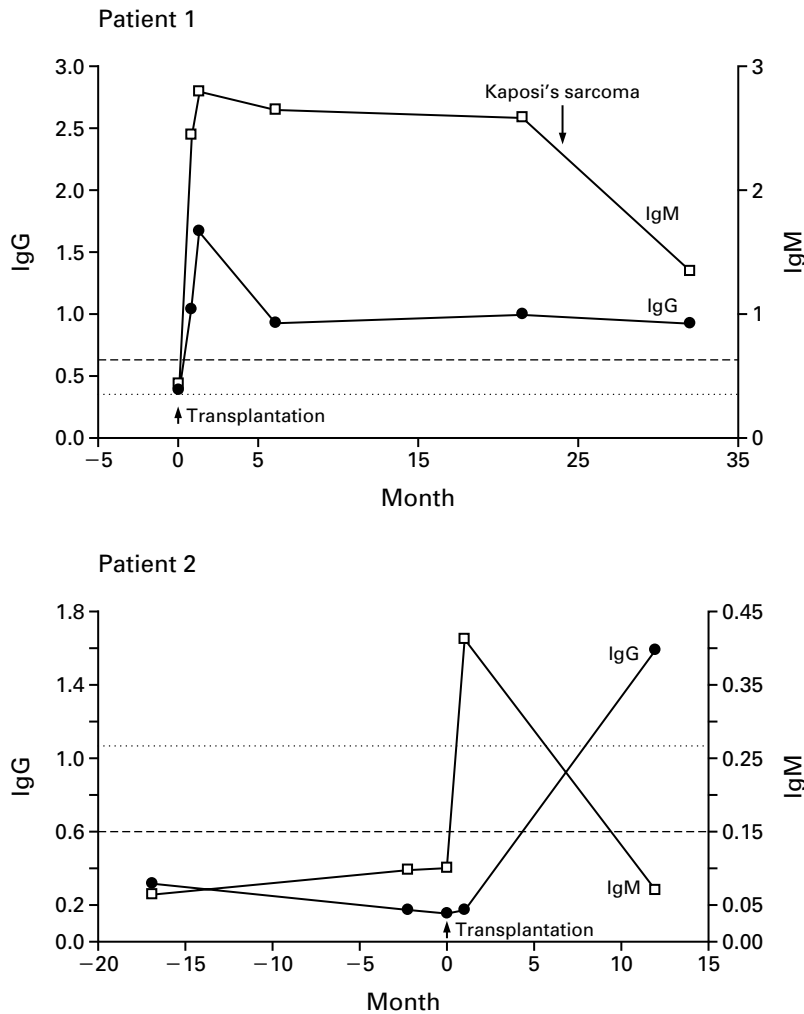


Figure 2. Representative Titers of IgM and IgG Antibody to HHV-8 in Two Transplant Recipients with Seroconversion within the First Year after Transplantation. The cutoff values are indicated by the dotted lines for IgM and the dashed lines for IgG.

eight patients (Patients 1, 3, 4, and 8) with early seroconversion had not received any blood products during the surgical procedure or within the first year after transplantation (Table 2). Thus, the early increase in IgM antibodies to HHV-8 after transplantation strongly suggests the occurrence of donor-transmitted HHV-8 infection. Patients 5 and 10 seroconverted more than four months after transplantation; therefore, a route of infection other than donor transmission cannot be ruled out.

The IgG antibodies to HHV-8 appeared between 2 weeks and 12 months after transplantation, and the titers stayed positive in all 10 patients.

Serum samples from 5 living donors and 1 cadaveric donor from among the 25 donors for the transplant recipients with seroconversion (donors for Patients 3, 8, 14, 17, 23, and 24) were available, and

5 (83 percent) tested positive for IgG antibodies to HHV-8, further supporting the hypothesis of donor-transmitted HHV-8 infection (Table 2). In the case of one donor (that for Patient 23), a serum sample could be collected only five years after transplantation and was negative for HHV-8 on ELISA, immunofluorescence assay, and Western blotting. Serum obtained from Patient 23 five years after transplantation was also negative on all three assays, indicating either that the original post-transplantation result was a false positive or that in some cases seropositive persons can revert to seronegative. In a control group of eight transplant recipients who were negative for HHV-8 at the time of transplantation, the corresponding donor serum samples tested negative for HHV-8 and none of the recipients seroconverted within one year after transplantation.

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TABLE 2. CHARACTERISTICS OF THE 25 PATIENTS WITH SEROCONVERSION WITHIN THE FIRST YEAR AFTER TRANSPLANTATION.*

PATIENT No.	AGE (YR)/SEX	UNDERLYING DISEASE	TYPE OF DONOR	HHV-8 SEROLOGIC STATUS OF DONOR	CMV SEROLOGIC STATUS AT TRANSPLANTATION	CMV SEROCONVERSION AFTER TRANSPLANTATION	UNITS OF PACKED RED CELLS†	CORTICOSTEROID PULSES†	THERAPY FOR CORTICOSTEROID-RESISTANT GRAFT REJECTION	IMMUNOSUPPRESSIVE REGIMEN AT 1 YR	CREATININE LEVEL AT 1 YR	MALIGNANT CONDITIONS
					donor/ recipient		no.				μmol/ml‡	
1	41/M	Glomerulonephritis	Cadaveric	NT	+/-	Yes	0	15	OKT3	C, Aza	169	Disseminated Kaposi's sarcoma; bone marrow plasmacytosis (25 percent)
2	61/F	Pyelonephritis	Cadaveric	NT	-/-	—	2	9	OKT3	C, Aza, P	97	—
3	37/F	Glomerulonephritis	Cadaveric	+	+/+	—	0	0	None	C	82	Anal squamous-cell carcinoma
4	33/M	Alport's syndrome	Cadaveric	NT	-/-	—	0	0	None	C	126	—
5	67/F	Analgesic nephropathy	Cadaveric	NT	+/-	Yes	4	9	OKT3	C	91	—
6	31/M	Reflux nephropathy	Cadaveric	NT	+/-	No	2	3	ATG	C	87	—
7	48/M	Glomerulonephritis	Cadaveric	NT	+/-	Yes	4	3	None	C	148	Epithelial skin carcinoma
8	34/F	Pyelonephritis	Living (mother)	+	+/+	—	0	0	None	C	106	—
9	58/M	Glomerulonephritis	Cadaveric	NT	-/+	—	12	9	ATG, OKT3	C, Aza, P	123	Kaposi's sarcoma of skin
10	32/M	Glomerulonephritis	Living (sister)	NT	+/+	—	0	0	None	C	114	—
11	59/M	Cystic nephropathy	Cadaveric	NT	-/+	—	0	0	None	C	116	—
12	27/M	Glomerulonephritis	Living (mother)	NT	?/+	—	0	3	None	C, Aza, P	168	—
13	57/F	Cystic nephropathy	Cadaveric	NT	-/+	—	6	3	None	C, Aza, P	118	Adenocarcinoma of the uterus before transplantation
14	28/F	Diabetic nephropathy	Living (mother)	+	+/+	—	2	0	None	C	99	—
15	32/F	Diabetic nephropathy	Living (mother)	NT	+/-	Yes	0	12	ATG	C, Aza	114	—
16	54/M	Glomerulonephritis	Living (brother)	NT	+/+	—	0	0	None	C	112	—
17	21/M	Nephrosclerosis	Living (mother)	+	-/+	—	2	24	ATG, OKT3, plasmapheresis	C, Aza, P	339	—
18	18/M	Reflux nephropathy	Living (father)	NT	+/-	No	0	0	None	C	121	—
19	60/F	Analgesic nephropathy	Cadaveric	NT	-/+	—	6	15	ATG	None	Dialysis	—
20	49/F	Cystic nephropathy	Cadaveric	NT	-/+	—	0	0	None	C	52	—
21	48/M	Unclear	Living (unrelated)	NT	-/-	—	4	6	None	C	107	—
22	69/M	Analgesic nephropathy	Cadaveric	NT	-/-	—	4	0	None	C, P	138	Metastatic colorectal carcinoma
23	60/M	Analgesic nephropathy	Living (unrelated)	-§	-/-	—	?	3	None	C, Aza, P	140	Metastatic colorectal carcinoma
24	39/F	Pyelonephritis	Living (unrelated)	+	+/+	—	2	0	None	C	76	—
25	48/M	Reflux nephropathy	Living (unrelated)	NT	+/-	No	4	0	None	C	105	—

*Patients 3 and 7 had undergone one previous transplantation, and Patient 20 had undergone two previous transplantations. CMV denotes cytomegalovirus, NT not tested, C cyclosporine, Aza azathioprine, P prednisone, and ATG antithymocyte globulin.

†Values are for the first year after transplantation. A corticosteroid pulse consisted of 0.5 g of methylprednisolone.

‡To convert values for creatinine to milligrams per deciliter, divide by 88.4.

§A serum sample obtained five years after transplantation was negative for HHV-8.

Characteristics of the Patients with Seroconversion after Renal Transplantation

The underlying renal diseases of the 25 patients with documented seroconversion (10 women and 15 men; mean age, 44.4 years) are listed in Table 2. Twelve of these patients received an allograft from a living donor and 13 from a cadaveric donor. Eight of the living donors were relatives, and four were unrelated partners of the patients. Before transplantation 12 recipients were seronegative for cytomegalovirus, 7 of whom had received allografts from seropositive donors. Four of these seven patients became seropositive for cytomegalovirus within one year after transplantation (Table 2). Thirteen recipients received packed red cells (2 to 12 units) in the first year after transplantation. Details of the immunosuppressive regimen, corticosteroid pulses, therapy for corticosteroid-resistant rejection, and serum creatinine levels are given in Table 2. There was no difference in graft survival up to four years after transplantation between the patients who seroconverted within one year after transplantation and the entire group of patients, whether they were seronegative or seropositive at the time of transplantation (data not shown). Fifteen of the 25 patients (60 percent) with functioning grafts were receiving cyclosporine as their sole immunosuppressive agent at one year. One patient was on hemodialysis because of chronic rejection.

Kaposi's sarcoma developed in two patients (Patients 1 and 9) with HHV-8 seroconversion. Both patients received anti-CD3 (OKT3, or muromonab-CD3) therapy for corticosteroid-resistant graft rejection. HHV-8 was also demonstrated by the polymerase chain reaction in samples of Kaposi's sarcoma tissue from both patients (data not shown). In Patient 1, disseminated Kaposi's sarcoma developed 24 months after transplantation (Fig. 2), and the patient eventually died. Autopsy revealed Kaposi's sarcoma in the lung, intestine, and lymph nodes. Kaposi's sarcoma of the skin developed 26 months after transplantation in Patient 9. The lesions were excised and the dose of the immunosuppressive therapy was reduced; the patient showed no evidence of recurrent disease after two years of follow-up.

DISCUSSION

Recent serologic studies have all reported that the prevalence of antibodies to latent and lytic HHV-8 antigens in the general population of Western countries is low — between 0 and 8 percent^{7,16,18,20-22} — with the exception of one study, which reported antibodies to HHV-8 in up to 25 percent of the U.S. general population.²³ We found seroprevalences of 5 percent among blood donors, 7 percent in the HIV-negative heterosexual population, and 20 percent in the HIV-negative homosexual population in Switzerland.¹⁸ The seroprevalence of 6.4 percent

among patients with renal failure before transplantation in the current study therefore is an accurate reflection of the seroprevalence of HHV-8 in our low-risk population. The change in seroprevalence from 6.4 percent before transplantation to 17.7 percent one year after transplantation suggests the occurrence of a new infection in a considerable number of these patients. An increase in the seroprevalence due to a possible cross-reaction with cytomegalovirus can be ruled out, since our ELISA does not detect other herpesviruses.¹⁸

Our data provide strong evidence of transmission of HHV-8 infection from the donor through the allograft. First, in the case of 6 of the 25 graft recipients who seroconverted, serum samples from the corresponding donors were available and 5 tested positive for HHV-8, whereas in a control group of 8 patients who were seronegative at the time of transplantation and who received allografts from HHV-8-negative donors, none seroconverted within one year. Second, the titer of IgM antibodies to HHV-8 increased within the first few months after transplantation in 8 of 10 patients for whom sequential serum samples were available for examination. Third, the possibility that HHV-8 was transmitted through blood products could be ruled out in four of the eight patients with early increases in IgM antibody titers (two of whom received an allograft from confirmed seropositive donors), since these patients did not receive blood products during the surgical procedure or within the first year after transplantation. Assuming that HHV-8 is transmitted by donor organs and that the seroprevalence of HHV-8 in the general population is between 0 and 8 percent, a doubling of the seroprevalence can be expected after transplantation. The observed increase in seroprevalence from 6.4 to 17.7 percent within the first year after transplantation is only slightly higher than predicted, suggesting that a few patients became infected by other routes (e.g., blood products or sexual contact).

Parravicini et al.²⁴ reported that 10 of 11 Italian recipients of renal allografts in whom Kaposi's sarcoma developed were seropositive for HHV-8 before transplantation and concluded that Kaposi's sarcoma among transplant recipients is primarily due to the reactivation of the virus during immunosuppression. Since several of their patients were apparently born in southern Italy, their conclusion may be correct with respect to patients from areas where HHV-8 is endemic. Our data suggest that in regions in which the virus is not endemic, transplantation-associated Kaposi's sarcoma is often transmitted from the donor.

In our study, both patients in whom Kaposi's sarcoma developed had severe immunosuppression as a result of treatment with monoclonal antibodies to T lymphocytes for corticosteroid-resistant graft rejection. This finding is consistent with results in the

literature, which suggest that the risk of Kaposi's sarcoma in transplant recipients depends on the severity of immunosuppression.¹⁵

In conclusion, our data show that HHV-8 is transmitted by donor organs. Patients who become seropositive for HHV-8 after transplantation are at risk for Kaposi's sarcoma, especially if they are severely immunosuppressed. Such patients therefore need close clinical follow-up.

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