MAJOR ARTICLE

Management and Outcomes after Multiple Corneal and Solid Organ Transplantations from a Donor Infected with Rabies Virus

T. Maier,[1,a] A. Schwarting,[2,a] D. Mauer,[4] R. S. Ross,[5] A. Martens,[6] V. Kliem,[7] J. Wahl,[3] M. Panning,[8] S. Baumgarte,[9] T. Müller,[10] S. Pfefferle,[8] H. Ebel,[1] J. Schmidt,[11] K. Tenner-Racz,[8] P. Racz,[8] M. Schmid,[4] M. Strüber,[6] B. Wolters,[5] D. Gotthardt,[12] F. Bitz,[7] L. Frisch,[3] N. Pfeiffer,[3] H. Fickenscher,[13,b] P. Sauer,[12] C. E. Rupprecht,[14] M. Roggendorf,[5] A. Haverich,[6] P. Galle,[2] J. Hoyer,[1] and C. Drosten[8,b]

[1]Department of Internal Medicine and Nephrology, University of Marburg Medical Centre, Marburg, Departments of [2]Medicine and [3]Ophthalmology, University of Mainz Medical Centre, Mainz, [4]German Organ Donor Procurement Organisation, Neu Isenburg, [5]Institute of Virology, University of Essen Medical Centre, Essen, [6]Clinic for Cardiac, Thoracic, Transplantation and Vascular Surgery, Hannover Medical School, Hannover, [7]Nephrological Centre of Lower Saxony, Hannoversch-Münden, [8]Bernhard Nocht Institute for Tropical Medicine and [9]Institute for Hygiene and Environmental Medicine, Hamburg, [10]Friedrich-Loeffler Institute–Federal Research Institute for Animal Health, Wusterhausen, and [11]Department of Surgery, [12]Liver Transplantation Section, Department of Medicine, and [13]Department of Virology, University of Heidelberg Medical Centre, Heidelberg, Germany; and [14]Rabies Section, Centers for Disease Control and Prevention, Atlanta, Georgia

Background. This article describes multiple transmissions of rabies via transplanted solid organ from a single infected donor. The empirical Milwaukee treatment regimen was used in the recipients.

Methods. Symptomatic patients were treated by deep sedation (ketamine, midazolam, and phenobarbital), ribavirin, interferon, and active and passive vaccination. Viral loads and antibodies were continuously monitored.

Results. Recipients of both cornea and liver transplants developed no symptoms. The recipient of the liver transplant had been vaccinated ~20 years before transplantation. Two recipients of kidney and lung transplants developed rabies and died within days of symptomatic disease. Another kidney recipient was treated 7 weeks before he died. The cerebrospinal fluid viral load remained at constant low levels (<10,000 copies/mL) for ~5 weeks; it increased suddenly by almost 5 orders of magnitude thereafter. After death, no virus was found in peripheral compartments (nerve tissue, heart, liver, or the small intestine) in this patient, in contrast to in patients in the same cohort who died early.

Conclusions. Our report includes, to our knowledge, the longest documented treatment course of symptomatic rabies and the first time that the virus concentration was measured over time and in different body compartments. The postmortem virus concentration in the periphery was low, but there was no evidence of a reduction of virus in the brain.

Rabies is an acute and invariably fatal form of viral encephalitis. Rabies virus, 1 of at least 7 species in the genus Lyssavirus, Family Rhabdoviridae, is acquired from infected animals by bites, scratches, and mucous membrane exposure [1]. If a history of bites does not exist, the disease is easily mistaken for other neurological or psychiatric conditions.

Transmission of rabies through corneal transplants has been described at least 8 times [2–4]. To our knowledge, transmission through solid-organ transplantation has been reported only once, when in 2004, four American patients received solid-organ transplants and vascular grafts from a donor whose rabies infection went undetected [5, 6]. We report a second similar event that occurred 9 months later in Germany, when 3 of 6 recipients of corneas or solid organs developed rabies. These patients underwent treatment using a neuroprotective and, hypothetically, antiviral regimen applied in a surviving patient with rabies in Milwaukee, Wisconsin [7]. Because no experiences with the regimen in other

Received 8 September 2009; accepted 7 December 2009; electronically published 5 March 2010.

[a] T. Maier and A. Schwarting contributed equally to this study.

[b] Present affiliation: Institute for Infection Medicine, University of Kiel, Kiel, Germany (H.F.); and Institute of Virology, University of Bonn Medical Centre, Bonn, Germany (C.D.).

Reprints or correspondence: Christian Drosten, Institute of Virology, University of Bonn Medical Centre, Sigmund Freud Str 25, 53105 Bonn, Germany.

Clinical Infectious Diseases 2010;50:1112–1119

1058-4838/2010/5008-0005$15.00
DOI: 10.1086/651267

patients were then available, 1 patient was treated over an unusually long course of 7 weeks with continuous monitoring of viral load in the cerebrospinal fluid (CSF).

PATIENTS, MATERIALS, AND METHODS

Patients. The organ donor was a 26-year-old woman who had been hospitalized for evaluation of an altered mental status with episodes of lethargy and aggressiveness. She had been seen 2 days earlier by a neurologist because of headaches and had reportedly consumed cocaine, amphetamines, and MDMA (3,4-methylendioxy-N-methylamphetamine) shortly before hospitalization. Neurological examination, cerebral magnetic resonance imaging (MRI), and CSF analysis showed no abnormalities. Within 1 day, she developed respiratory distress and fever. She received antibiotic therapy, catecholamines, and ventilator therapy after sudden cardiac arrest, which occurred during the same day. Computer tomography revealed massive cerebral edema. Examination of a CSF specimen obtained 5 days after the first sample revealed 700 cells/μL, protein concentration of 1.3 g/L, a glucose level of 0.7 g/L, and lactate concentration of 7 mmol/L. Culture of CSF and blood samples yielded no bacteria or fungi. The results of polymerase chain reaction (PCR) of CSF samples were negative for herpes simplex viruses 1 and 2, varicella zoster virus, cytomegalovirus, enteroviruses, flaviviruses, and alphaviruses. Brain death was confirmed in a subsequent neurological examination. The donor's family consented to a multiorgan extraction. Histories of all organ recipients are summarized in Table 1.

Laboratory procedures. Histological examination followed standard procedures. Antibodies were tested by enzyme immunoassay (Platelia Rabies II Kit; Bio-Rad). Virus-neutralizing antibodies were determined by the rapid fluorescent focus inhibition test [8]. Heminested reverse-transcription (RT) PCR used primers adapted from a published protocol [9]. The full genome sequence of rabies virus was determined directly from sputum of recipient 4 (GenBank AY956319).

Empirical treatment. Details on treatment of a patient who survived rabies were made available prior to publication by Dr Willoughby (University of Wisconsin; Milwaukee) [7]. Relatives and patients were informed that treatment was highly likely to fail and that severe disability had to be expected even in the case of recovery. Treatment was preferred over palliative care in all cases.

RESULTS

Laboratory Confirmation and Chains of Transmission

In the course of a routine look-back procedure on day 45 after transplantation, similarity of symptoms of recipient 4 with those of the donor was notified. Similar findings were also confirmed in recipients 5 and 6. Archived paraffin-embedded samples of the donor's brain stem were reexamined by hematoxilin-eosin staining. In addition to massive perivascular infiltrations, which had already been noticed upon initial examination, eosin-dense Negri bodies were seen in ganglion cells (Figure 1). Electron microscopy of sections from various brain regions showed virus particles with typical bullet-shaped rhabdovirus morphology. To confirm the suspicion of rabies specifically, monoclonal antibodies were used for staining of virus antigen (Figure 1). No positive RT-PCR results were seen for saliva and skin biopsy specimens obtained from recipients 1 and 2, as well as with saliva and corneal swab samples obtained from recipient 3. Clear amplification was achieved in bronchoalveolar lavage, sputum, and bronchial secretion samples obtained from recipient 4; in saliva samples from recipient 5; and in sputum and corneal swab specimens obtained from recipient 6. Virus RNA was also detected in formalin-fixed brain of the donor by adapted nested RT-PCR. All PCR product sequences were 100% identical, proving transmission. Virus was isolated in cell cultures for recipients 4 and 6. Phylogenetic comparison with the archival database at the Centers for Disease Control and Prevention revealed closest homology (93% nucleocapsid nucleotide identity) with a rabies virus from an American patient bitten by a dog in India [11]. Meanwhile, the travel history of the donor had been reconstructed more precisely and matched this observation: a fellow traveler who had not been available for interviews before remembered the donor had been bitten by a stray dog in India.

Clinical Courses after Diagnosis

All recipients were contacted immediately when findings in the donor were communicated on day 46 after transplantation. Postexposure prophylaxis (PEP) was started immediately for all recipients, comprising a single 20-IU/kg dose of human antirabies hyper-immunoglobulin (HRIG; Berirab; Behring) and 2.5 IU of purified chicken embryo cell vaccine (PCECV; Rabipur, Behring) intramuscularly after 0, 3, 7, 14, and 28 days. After completion of PEP, recipients 1 and 2 showed titers of rabies virus–specific antibodies of 1.0 and 6.0 IU/mL, respectively. Corneal grafts were removed and replaced in both recipients. Rabies virus RT-PCR repeatedly yielded negative results for the removed corneas. Both patients had not developed symptoms to date.

Recipient 3. Recipient 3 was asymptomatic when he received PEP. Surprisingly, virus-neutralizing antibodies were already detectable at 0.4 IU/mL in an archived pretransplantation blood sample. Neutralization titers increased to 29.5, 30.4, and >60 IU/mL on days 46, 53, and 61, respectively. His mother recalled that he had been vaccinated against rabies during childhood, ~20 years ago. Ribavirin (800 mg/day) and pegylated interferon-α-2b (100 μg per week) was administered in spite of these findings because of the artificial route of rabies virus exposure. Liver enzyme levels increased after 3 weeks, and

Table 1. Characteristics of Transplant Recipients Who Received Transplants from a Rabies Virus–Infected Donor

	Transplant recipient					
Characteristic	1	2	3	4	5	6
Age, years	58	39	26	46	72	47
Sex	Male	Female	Male	Female	Male	Male
Primary disease(s)	Corneal decompensation after 31-year contact lens history, aphakia	Congenital glaucoma, decompensation of a 6-year-old corneal graft	Primary sclerosing cholangitis	Bronchiolitis obliterans syndrome, primary heart/lung transplantation 17 years earlier	Chronic glomerulonephritis, 10-year history of hemodialysis	Type 1 diabetes, end stage renal disease, 9-month history of peritoneal dialysis
Organ or tissue transplant	Cornea	Cornea	Liver	Lung	Kidney	Kidney and pancreas
Immunosuppressive therapy	None	None	Cyclosporin A (3.5–5.3 mg/kg per day)	Tracrolimus (0.5 mg per day), mycophenolatmofetil (2 g per day), prednisolon 10 (mg per day)	Cyclosporin A (3.5–5.3 mg/kg per day), mycophenolatmofetil (2 g per day), prednisolon (10–15 mg per day)	Cyclosporin A (3.5–5.3 mg/kg per day), mycophenolatmofetil (2 g per day), prednisolon (20 mg per day), antithymocyte globulin (2 mg per day; 4 doses)
Postoperative course	Uncomplicated	Uncomplicated	Uncomplicated, normal MRI findings	Tracheostoma; not discharged after transplantation; unresponsiveness/lethargy from day 20 and renal failure on day 30; uypersalivation, dysphagia, abnormal pupil reactions from day 42; normal CSF findings and normal MRI, CT, and EEG findings; normal virological findings, except IgM anti-CMV	Discharged after 21 days; dizziness and dysphagia from day 35; readmission and rapid neurologic deterioration from day 40; generalized seizure and paraparesis on day 41; respiratory failure/intubation on day 43; no meningism, and normal CSF and virological findings	Discharged after 29 days; lymphocyte count after receipt of anti-thymocyte globulin, 230 cells/μL ($CD4^+$ cell count, 48 cells/μL; hydrophobia starting around day 35; readmission on day 43 with obstipation, altered mental status, and agitation; CSF data on day 44: 13 cells/μL; glucose level, 68 mg/dL; protein level, 560 mg/dL; lactate level, 2.63 mmol/L; normal virological findings
Outcome	Survived	Survived	Survived	Died	Died	Died

NOTE. CMV, cytomegalovirus; CSF, cerebrospinal fluid; CT, computed tomography; EEG, electroencephalography; IgM, immunoglobulin M; MRI, magnetic resonance imaging.

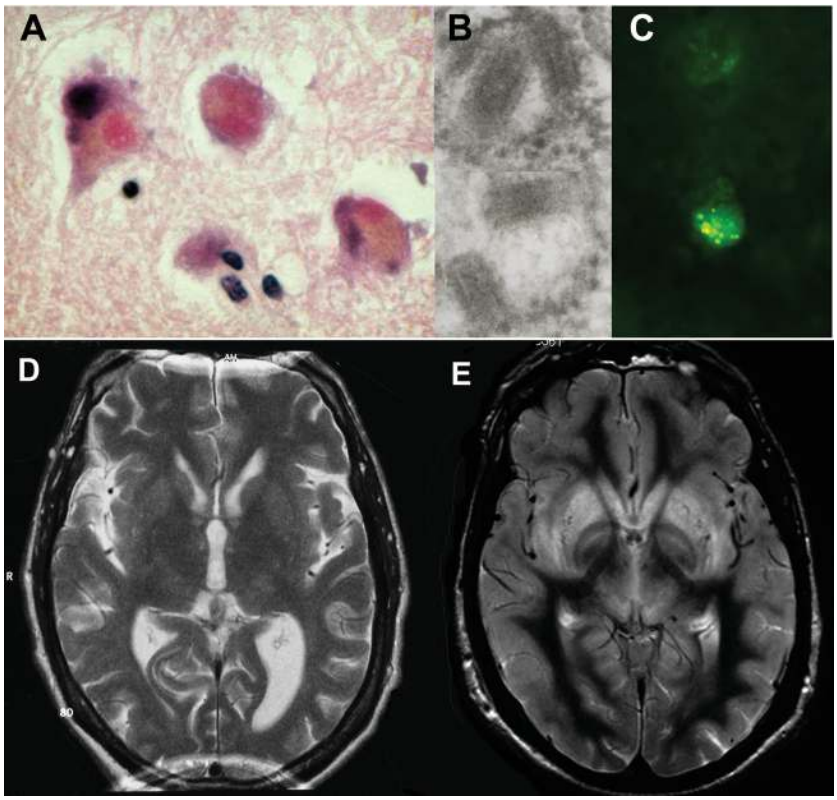

Figure 1. Pathology and radiology findings. *A–C,* Findings in brain stem sections of the organ donor. *A,* Negri bodies *(red)* in paraffinized, formalin-fixed tissue stained with hematoxilin and eosin. *B,* Budding rabies virions, as seen in electron microscopy on deparaffinized brain sections treated with phosphotungstic acid; *C,* Direct fluorescent staining of rabies virus antigen in deparaffinized, acetone-fixed brain by fluorescein isothiocyanate-labeled monoclonal antibodies against rabies virus nucleoprotein. *D* and *E,* Findings in T2-weighted magnetic resonance imaging in recipient 6 one week after laboratory confirmation of rabies (*D;* no significant findings and weeks after confirmed rabies diagnosis *(E;* hyperintensity of grey substance, signal change of basal ganglia and subthalamic nuclei, and general swelling of cortex).

biopsy demonstrated rejection. Ribavirin and interferon were stopped, and short-term, high-dosage immunosuppressive therapy was initiated. Liver enzyme levels reverted to normal within 18 days. The patient is healthy to date.

Recipient 4. Recipient 4 had already received PEP on day 45 on the basis of clinical suspicion of rabies, and an additional 20-IU/kg dose HRIG was given on day 47. Deep sedation was induced with ketamine (100–125 mg/h) and midazolam (15–20 mg/h). Antiviral therapy was initiated with interferon-α at 1.5Mio IE subcutaneously and intravenous ribavirin (loading dose, 200 mg). On day 47 after transplantation, the patient developed atrioventricular block and received an intravenous pacemaker. She died of cardiac arrest 2 days later without ever developing virus neutralizing antibodies detectable by enzyme immunoassay or neutralization test.

Recipient 5. Recipient 5 started PEP on the basis clinically suspected rabies on day 45 after transplantation After laboratory confirmation, additional passive vaccination was administered at 25 IU/kg of HRIG per day until day 51. Ribavirin was administered at 4–8 mg/kg every 6 h after an initial loading dose of 20 mg/kg. Interferon-α (3,000,000 IU) was given subcutaneously every second day. Amantadine (200 mg per day) was added on day 50. The patient died of sudden cardiac arrest on day 52 after transplantation. Antibodies against rabies virus were not detectable by enzyme immunoassay in serum samples obtained on days 45, 47, and 51 after transplantation.

Recipient 6. Recipient 6 worsened with panic attacks, hypersalivation, and respiratory distress from day 45 after transplantation, requiring intensive care. MRI revealed no pathological lesions (Figure 1). Anti-thymocyte globulin and mycophenolatmofetil were stopped, and the cyclosporin A dose was reduced stepwise over 4 days. Deep sedation was induced with midazolam (4–8 mg per hour) starting on day 47. Ketamine and phenobarbital were added at 25–100 mg per hour and 8–16 mg per hour, respectively, yielding complete burst suppression in episodes of 10–25 s on an electroencephalograph (EEG).

In addition to conventional PEP, HRIG was continued after the first dose at 27 IU/kg per day. Antiviral treatment was started on day 47 with ribavirin (22 mg/kg 4 times per day over 3 days and then 11 mg/kg every 8 h). Interferon-α was given subcutaneously at 180 μg (Pegasys; Chiron) on day 46 after transplantation, 5,000,000 IU (Roferon; Roche) was given

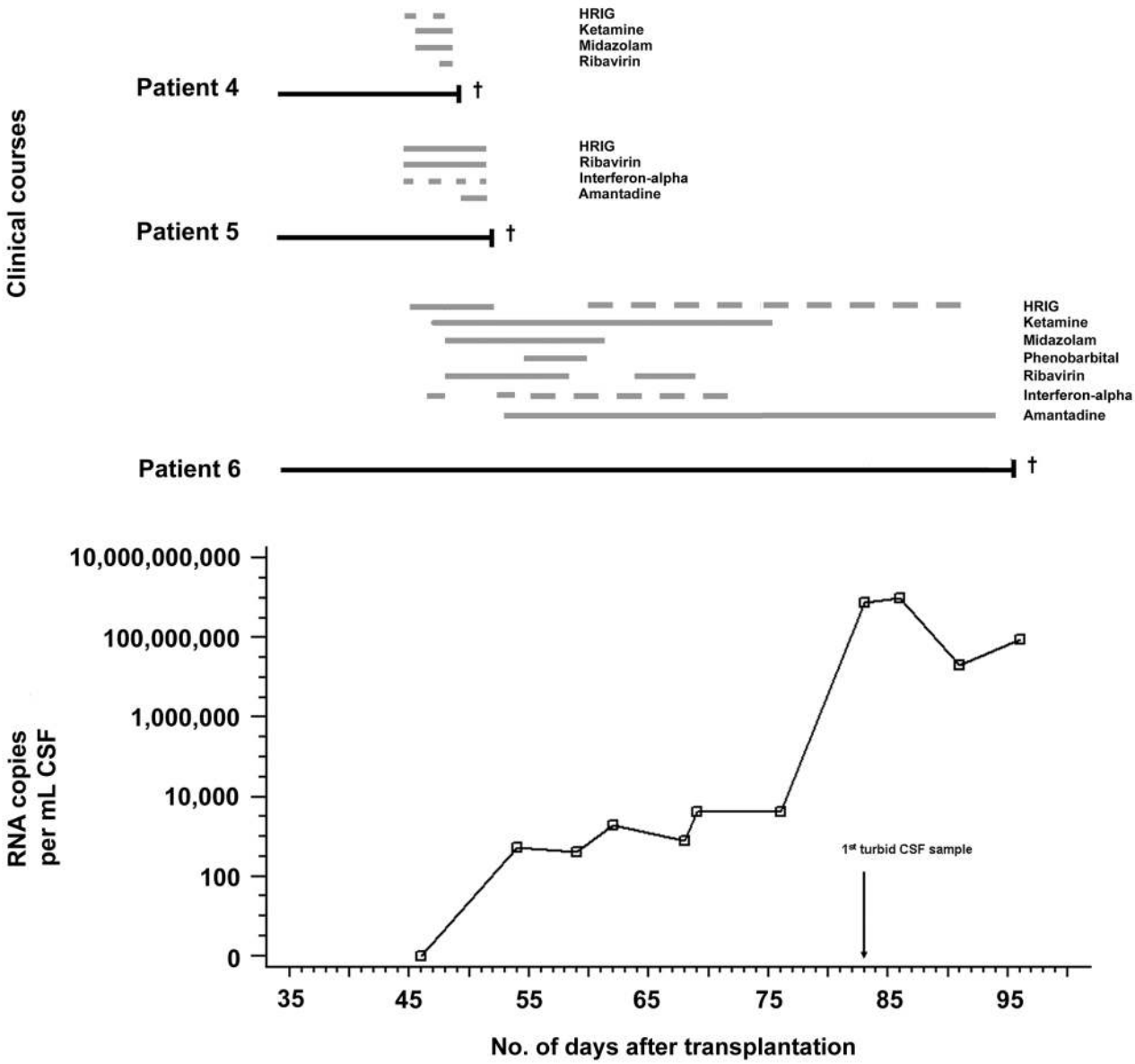

Figure 2. Clinical courses and overview of therapy for recipients 4 (kidney transplant recipient), 5 (heart and lung transplant recipient), and 6 (spleen and kidney transplant recipient), who died of rabies. *Bottom,* Virus RNA concentration over time as determined by real-time reverse-transcription polymerase chain reaction of a cerebrospinal fluid (CSF) specimen obtained from recipient 6. The assay employed oligonucleotides AGAAGGGAATTGGGCTTTGAC, AGATGCATGCTCGGGAACA, and FAM-ATGGGGTCCCTCGTCAGTTCCATT-TAMRA in the AY956319 nucleocapsid gene. The limit of detection was characterized by probit analysis on in vitro transcribed cRNA, with 185 copies of RNA/mL of CSF being detectable at >95% probability. HRIG, human anti-rabies hyper-immunoglobulin.

on day 53, and 3,000,000 IU was given every 3 days starting from day 56. Amantadine (4–16 mg per hour) was added on day 53.

Virus RNA concentration in CSF on day 52 was 521 copies/mL. Virus was undetectable in a retrospective CSF sample drawn 1 week earlier. Because comparable data from other patients were not available in the literature, viral loads were determined in stored CSF samples from recipients 4 and 5 from days 45 and 46 after transplantation, respectively, a few days before each respective patient's death. CSF viral loads were 13,000 and 89,125 copies/mL, respectively. A prognostic advantage was therefore assumed for recipient 6, supporting decisions to continue treatment. The T lymphocyte count at this time was 191 cells/μL, with a $CD4^+$ cell count of 38 cells/μL. The virus-neutralizing antibody titer level on day 50 was 0.25 IU/mL, increasing to 0.56 IU/mL 2 days later. To probe a possible natural immune response, HRIG was discontinued. However, on days 54 and 58, no further increase in neutralization

titer was seen (0.56 and 0.54 IU/mL, respectively) (Figure 2), and passive immunization was reinitiated on day 60 with HRIG (27 IU/kg every 2 days).

Because of described low penetration of ribavirin into the central nervous system [10], ribavirin was administered intrathecally at 40 mg per day on days 52–58 and at 20 mg per day on days 63, 65, 67, 70, and 77. To provide potential protection against the grave adverse effects of ribavirin during high-dosage therapy [7], coenzyme Q10, N-acetylcysteine (0.6 g per day), ascorbic acid (1 g per day), and tocopherol (0.1 g per day) were added. Interferon-α was also administered intrathecally at 1.5×10^6 IU on days 54 and 65. Cardiac arrest and severe arrhythmia on day 52 were managed with catecholamines and a temporary pacemaker device. Midazolam and phenobarbital were discontinued because of prolonged suppression episodes noted by EEG on days 55 and 56, respectively. Ribavirin was discontinued on days 59–64 and after day 69 because of an elevation in liver enzyme levels. The T lymphocyte count on day 62 was only marginally improved, at 245 cells/μL ($CD4^+$ cell count, 42 cells/μL). Virus-neutralization titer levels stayed constant at 0.54–0.6 IU/mL in 5 subsequent determinations conducted during days 61–73.

After 3 weeks of therapy, the patient developed severe diabetes insipidus. Pituitary, thyroid, and adrenal function were perturbed from day 61 onward, requiring desmopressin at 0.5 μg per day on average, until the end of the course. Hypothermia was observed during the fourth week. Up to the fifth week (day 76 after transplantation), when ketamine had to be discontinued because of excessive burst suppression in EEG, only a very slow increase in the CSF viral load was seen. A value of 4300 copies/mL was never exceeded up to this point (Figure 2). MRI revealed only very mild enhancement in basal ganglia and putamen. No abnormal findings were observed in CSF cytology and microscopy up to this point.

At the beginning of the sixth week of therapy (day 80 after transplantation), MRI revealed hyperintensity in the gray substance and a signal change in the basal ganglia and subthalamic nuclei. General swelling of the cortex was also evident (Figure 1). MRI performed 2 days later showed aggravation of lesions with increasing edema and swelling. Abruptly on day 83 after transplantation, CSF showed strong pleocytosis, with macrophage-like cells containing pycnotic nuclei. The viral load increased by >4 $\log_{10}$ intervals, reaching a peak value of 9.96×10^8 copies/mL of CSF (Figure 2). The blood lymphocyte count had increased to 811 cells/μL in a determination on day 90, with a $CD4^+$ cell count of 203 cells/μL. EEG did not recover when sedation was discontinued on day 93. A Tc-hexamethylpropyleneamine oxmie (HMPAO)–Te99-scan suggested stall of brain metabolism. EEG and apnea test were indicative of brain death on day 95 after transplantation, 64 days after the onset of hydrophobia as the first recorded symptom of infection. All therapeutic and supportive measures were discontinued. Cardiac arrest occurred on the same day. Table 2 provides virus concentrations in selected postmortem organs of transplant recipients 4–6.

Table 2. Virus Concentration in Selected Organs

	Transplant recipient, RNA copies/mg of tissue		
Sample	4	5	6
Central nervous system	1.2×10^9	6.1×10^9	2.3×10^9
Peripheral nerve	3.8×10^6	1.3×10^6	0
Small intestine	2.8×10^5	NA	0
Liver	9.2×10^3	NA	0
Heart	4.2×10^3	NA	0

NOTE. NA, material not available.

DISCUSSION

This second report on transmission of rabies by solid-organ transplantation emphasizes the role of travel histories in donor eligibility screening. Due to associated delays and poor predictive value in a setting of nonendemicity, screening for rare agents (eg, arboviruses) is not mandatory in organ donors in Germany. Even in the case of rabies, where testing could be done after transplantation due to the option of PEP, the low incidence of disease has thus far prevented mandatory testing of donors. This may have to be changed specifically for donors with altered mental state.

Interesting implications can be made from the cases of our surviving recipients. The fortunate case of our liver recipient provides proof of protection against transplantation-associated rabies even after a long interval since vaccination. Vaccination dated back ~20 years, and the inoculum received from the transplant organ was probably high. For comparison, the postmortem virus concentration in the liver of recipient 4 was 9,200,000 copies/g of tissue. It is highly important in this context that an alternative way of introduction of rabies virus into the central nervous system via the blood has recently been demonstrated [12]. Because transplanted tissue is deprived of direct nerval input for many months [13], it is not unlikely that virus in our cases had to use a hematogenous route to enter the central nervous system [12]. Protection by neutralizing antibodies might therefore be particularly effective in transplant recipients.

Fortunate courses were also seen in the two recipients of corneas. Since both of them were not vaccinated before transplantation, their virus inoculum must have been below an infectious dose. It is very likely that corneas contained rabies virus immediately after donation because virus is secreted in lacrimal glands. Its concentration may have been reduced during subsequent organ culture in which tissues were kept for

more than 24 h, as usual in Germany. This would be in concordance with studies on hepatitis B and C viruses, which were also found at low or undetectable levels in precultured corneas form viremic donors [14].

Three of our organ recipients died in spite of maximal efforts. One patient underwent the longest documented course of treatment of rabies. The essential treatment protocol was provided to us by colleagues from the University of Wisconsin prior to publication [7]. It involved ketamin due to its hypothetical inhibitory effect on RNA transcription and N-methyl D-aspartate (NMDA)–antagonistic function that might limit viral spreading in tissue [10, 15]. Similar hypothetical functions were ascribed to amantadine and ribavirin [7]. Early availability of this protocol was an important factor in clinical decision making, as was the initial finding in patient 6 of undetectable virus RNA in CSF and its continued low concentration over the ensuing course. On the other hand, our patients lacked significant factors promoting good outcome that were present in the case treated in Wisconsin [7]. This patient was a young and otherwise healthy girl diagnosed at a very early stage of symptoms. Antibodies were present already at the initial diagnosis, in contrast to our patients with underlying disease and strong therapeutic immunosuppression. Moreover, the pathogenesis of silver haired bat rabies virus, which the patient would probably have contracted might differ from that of street rabies viruses [16–18]. Some bat viruses appear to replicate in vitro at a certain efficiency in cells outside the central nervous system such as fibroblasts [17], which could promote in vivo a more efficient natural immune response through antigen presentation. The potential involvement of blood passage in the pathogenesis of silver haired bat rabies virus infection [12] might add to the effectiveness of immune response. The Wisconsin case might thus have been in an extraordinary favorable condition for success of invasive intensive care treatment.

Because we did not expect efficient natural immune response in our patients, post exposure vaccination was included in our modified treatment regimen. Extended treatment also involved the provision of interferon alpha and substances with hypothetical antiviral activity in CSF. It should be noted that real-time PCR showed no increase of virus concentration in CSF over >5 weeks. The viral load appeared to increase only after ketamine was discontinued. However, caution is necessary in interpreting this finding. More recent experimental data could not confirm any protective effect of ketamine on mouse neural cells infected with rabies virus [19]. Our actual reason for stopping ketamine was an extension of suppression episodes in EEG, which in turn may have been caused by viral neurotoxicity or by immune-mediated phenomena. Changes in MRI, the sudden appearance of turbid CSF, and the fact the terminal CSF viral load corresponded to brain virus concentration together suggest an immune-mediated lysis of brain tissue along with a breakdown of the brain-CSF barrier. Antibody response was weak and did not seem to correlate with clinical changes, and it was thus not likely to have caused lysis. On the contrary, the terminal increase in viral load went along with a clear reconstitution of lymphocyte counts, and macrophage-like cells appeared in CSF. A late cellular immune response may thus have led to destruction of central nervous tissue rather than elimination of the virus. Indeed, experimental data suggest that primary neuronal degeneration is caused by the virus, but T cells are then recruited to neural tissue and cause strong secondary degeneration [20]. In the mouse model, virus clearance and survival depend on the presence of both a sufficient level of virus neutralizing antibodies and a cellular inflammatory reaction early enough during infection [20, 21].

It is interesting to note that, in recipient 6, no virus RNA could be detected in postmortem peripheral nerve and intestinal tissue specimens. This was contrary to findings in recipients 4 and 5, in whom virus was detected at high concentrations in peripheral nerve and organ specimens, compatible with centrifugal spread in the symptomatic phase. It appears possible that immune response—or, less likely, treatment—might have influenced the virus in the periphery, whereas the central nervous compartment could not be reached. However, we have no means to verify this assumption. Another possible explanation would be stagnation of centrifugal virus spread after a long course of disease.

This report, as well as more recent observations in other patients, attenuate expectations regarding therapeutic intervention in human rabies [22–24]. More basic research leading to novel immunotherapies or antiviral drugs are urgently needed [25, 26].

Acknowledgments

We are grateful to Rodney Willoughby, Medical College of Wisconsin, for advice and assistance with treatment protocols. We thank the following individuals for helpful discussion and suggestions: Eberhard Weihe, Marburg; Eberhard Walters, Marburg; Siegfried Biehn, Marburg. The Bernhard Nocht Institute acts for the German Ministry of Health as the National Reference Centre for Imported Infectious Diseases. The Department of Virology, University of Essen, acts for the German Ministry of Health as the consiliar laboratory for rabies.

Potential conflicts of interest. All authors: no conflicts.

References

1. Rupprecht CE, Hanlon CA and Hemachudha T. Rabies re-examined. Lancet Infect Dis **2002**; 2:327–343.
2. Anonymous. Human-to-human transmission of rabies via corneal transplant–Thailand. MMWR Morb Mortal Wkly Rep **1981**; 30:473–474.
3. Gode GR, Bhide NK. Two rabies deaths after corneal grafts from one donor. Lancet **1988**; 2:791.
4. Houff SA, Burton RC, Wilson RW, et al. Human-to-human transmission of rabies virus by corneal transplant. N Engl J Med **1979**; 300:603–604.
5. Srinivasan A, Burton EC, Kuehnert MJ, et al. Transmission of rabies virus from an organ donor to four transplant recipients. N Engl J Med **2005**; 352:1103–1111.

6. Kusne S, Smilack J. Transmission of rabies virus from an organ donor to four transplant recipients. Liver Transpl **2005**; 11:1295–1297.
7. Willoughby RE Jr, Tieves KS, Hoffman GM, et al. Survival after treatment of rabies with induction of coma. N Engl J Med **2005**; 352:2508–2514.
8. Rabies. In: Manual of diagnostic tests and vaccines for terrestrial animals. 5th ed. Paris: World Organisation for Animal Health (OIE), **2004**: 313–315.
9. Heaton PR, Johnstone P, McElhinney LM, Cowley R, O'Sullivan E, Whitby JE. Heminested PCR assay for detection of six genotypes of rabies and rabies-related viruses. J Clin Microbiol **1997**; 35:2762–2766.
10. Jackson AC, Warrell MJ, Rupprecht CE, et al. Management of rabies in humans. Clin Infect Dis **2003**; 36:60–63.
11. Centers for Disease Control and Prevention. Human rabies—California, 1992. MMWR Morb Mortal Wkly Rep **1992**; 41:461–463.
12. Preuss MA, Faber ML, Tan GS, Bette M, Dietzschold B, Weihe E, Schnell MJ. Intravenous inoculation of a bat-associated rabies virus causes lethal encephalopathy in mice through invasion of the brain via neurosecretory hypothalamic fibers. PLoS Pathog **2009**; 5:e1000485.
13. Arrowood JA, Minisi AJ, Goudreau E, Davis AB, King AL. Absence of parasympathetic control of heart rate after human orthotopic cardiac transplantation. Circulation **1997**; 96:3492–3498.
14. Sengler U, Reinhard T, Adams O, Gerlich W, Sundmacher R. Testing of corneoscleral discs and their culture media of seropositive donors for hepatitis B and C virus genomes. Graefes Arch Clin Exp Ophthalmol **2001**; 239:783–787.
15. Lockhart BP, Tordo N, Tsiang H. Inhibition of rabies virus transcription in rat cortical neurons with the dissociative anesthetic ketamine. Antimicrob Agents Chemother **1992**; 36:1750–1755.
16. Yan X, Prosniak M, Curtis MT, Weiss ML, Faber M, Dietzschold B, Fu ZF. Silver-haired bat rabies virus variant does not induce apoptosis in the brain of experimentally infected mice. J Neurovirol **2001**; 7: 518–527.
17. Morimoto K, Patel M, Corisdeo S, et al. Characterization of a unique variant of bat rabies virus responsible for newly emerging human cases in North America. Proc Natl Acad Sci U S A **1996**; 93:5653–5658.
18. Warrell MJ, Warrell DA. Rabies and other lyssavirus diseases. Lancet **2004**; 363:959–969.
19. Weli SC, Scott CA, Ward CA, Jackson AC. Rabies virus infection of primary neuronal cultures and adult mice: failure to demonstrate evidence of excitotoxicity. J Virol **2006**; 80:10270–10273.
20. Galelli A, Baloul L, Lafon M. Abortive rabies virus central nervous infection is controlled by T lymphocyte local recruitment and induction of apoptosis. J Neurovirol **2000**; 6:359–372.
21. Hooper DC, Morimoto K, Bette M, Weihe E, Koprowski H, Dietzschold B. Collaboration of antibody and inflammation in clearance of rabies virus from the central nervous system. J Virol **1998**; 72:3711–3719.
22. Hemachudha T, Sunsaneewitayakul B, Desudchit T, et al. Failure of therapeutic coma and ketamine for therapy of human rabies. J Neurovirol **2006**; 12:407–409.
23. McDermid RC, Saxinger L, Lee B, et al. Human rabies encephalitis following bat exposure: failure of therapeutic coma. CMAJ **2008**; 178: 557–561.
24. van Thiel PP, de Bie RM, Eftimov F, et al. Fatal human rabies due to duvenhage virus from a bat in Kenya: failure of treatment with coma-induction, ketamine, and antiviral drugs. PLoS Negl Trop Dis **2009**; 3:e428.
25. Jackson AC. Update on rabies diagnosis and treatment. Curr Infect Dis Rep **2009**; 11:296–301.
26. Wilde H, Hemachudha T, Jackson AC. Viewpoint: management of human rabies. Trans R Soc Trop Med Hyg **2008**; 102:979–982.