Instructive even after a decade: Complete results of initial virological diagnostics and re-evaluation of molecular data in the German rabies virus "outbreak" caused by transplantations

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ABSTRACT

In 2005, six patients in Germany received solid organs and both corneas from a donor with an unrecognized rabies infection. Initial virological diagnostics with the machinery available at the two national reference laboratories could quickly clarify the situation. Rabies virus antigen was detected in the organ donor's brain. In two of the three recipients with neurological alterations, intra vitam diagnosis was achieved by conventional RT-PCRs. Comparison of the phylogenetic relatedness of the different viral isolates proved transmission from the donor and, consequently, also established the diagnosis for the third patient. As indicated by the titre of neutralizing antibodies, the liver transplant recipient was protected from the lethal infection due to a vaccination against rabies virus, which he had received more than 15 years ago. All samples from the recipients of the corneas were invariably negative. Re-evaluation of the molecular data by real-time PCR did not lead to an improvement of intra vitam diagnosis but provided intriguing insights regarding the relative amounts of rabies virus RNA in different body fluids and peripheral organs. In saliva and skin, they were 250–200,000 times lower than in the infected patient's brains. Furthermore, in saliva samples taken serially from the same patient fluctuations by a factor of 160–500 were recorded. These findings highlight the problems of intra vitam diagnosis of rabies virus infections and make understandable why the virus can escape from all diagnostic attempts. Finally, in this context one should recall an almost trivial fact: Simple and appropriate postexposure prophylaxis could not only have saved the young organ donor's life but would also have prevented the whole transplantation-associated rabies "outbreak" in Germany.

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Introduction

During the past six decades, the clinical science of transplantation was one of the most fascinating and rapidly growing fields of medicine (Barker and Markmann, 2013). Generally, the benefits of transplantation for the patients in terms of an improved quality of life and an extension of life expectancy by far outweigh the undeniable risks to which transplant recipients are exposed. In this respect, viral infections are of particular concern, and there is a constant fear that certain "exotic" pathogens (Kaul, 2013; Waggoner et al., 2013), among them the West Nile virus (Iwamoto et al., 2003), the lymphocytic choriomeningitis virus (Fischer et al., 2006), and also the rabies virus, might be transferred to the recipients. The latter virus is mainly transmitted to humans by bites of infected animals in countries where the disease is enzootic and causes an almost uniformly fatal encephalitis (Bleck and Rupprecht, 2009; Hemachudha et al., 2013; Jackson, 2009; Mani and Madhusudana, 2013; Warrell and Warrell, 2004). Since the first successful kidney transplantation up to 2004 no more than eight incidents of human-to-human transmission of the rabies virus were recorded worldwide, and all these cases occurred among recipients of cornea grafts (Galian et al., 1980; Gode and

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Bhide, 1988; Houff et al., 1979; Javadi et al., 1996; Thongcharoen et al., 1981). In 2004 for the first time, four incidents of rabies infection in transplant patients could be ascribed to solid organ and vascular tissue grafting in the United States (Burton et al., 2005; Srinivasan et al., 2005). About one year later, in February 2005, in Germany the suspicion arose that six individuals, who had received organs and the corneas from a common donor, could have also been infected with the rabies virus by the transplants (Ross et al., 2005). The clinical courses of the recipients were reported in detail elsewhere (Maier et al., 2010; Vetter et al., 2011). This communication aims for the first time to provide the complete spectrum of virological findings initially raised in the two national reference laboratories for rabies virus infection and also includes the results of a re-evaluation of the primary molecular data.

Case reports

Transplant recipients

In February 2005, symptoms of altered mental status were recorded in three recipients of the lung, the two kidneys, and the pancreas from a common organ donor (patients 1, 2, and 3 in Table 1). All patients developed rapid neurological deterioration and signs of an unexplained acute encephalitis between 40 and 43 days after the transplantations. Simultaneous rabies postexposure prophylaxis (WHO, 2013) was started immediately after the onset of symptoms. The patients died on days 49, 52, and 95 after the transplantations, respectively (Maier et al., 2010).

The liver and both corneas of the same organ donor were grafted to three other individuals (patients 4, 5, and 6 in Table 1), who did not show any symptoms indicative of encephalitis. Both corneas had been kept for five days at 4 °C in the hypothermic storage medium Optisol GS (Armitage, 2008) prior to transplantation. Simultaneous rabies postexposure prophylaxis (WHO, 2013) was started in these patients on days 41 and 46 after the transplantations, respectively, and the cornea grafts were replaced. The three individuals were subjected to long-lasting follow-up investigations and never developed rabies (Vetter et al., 2011).

Organ donor

The 26-year old woman was seen by different physicians mainly due to psychiatric alterations. She had a history of drug abuse and reported that she had consumed cocaine, amphetamines, and also ecstasy shortly before she was admitted to the hospital. Analyses of her cerebrospinal fluid and MRT initially did not show any abnormalities. The patient's clinical condition worsened rapidly. She developed sudden cardiac arrest and, after resuscitation had failed, brain death was confirmed. Donor eligibility screening and testing in accordance with the then relevant national recommendations (Bundesärztekammer, 2005) did not reveal any contraindications to transplantation. Therefore, the lung, both kidneys, the pancreas, and both corneas were recovered, subsequently allocated to five transplant centres throughout Germany, and finally grafted to six recipients on 31st December 2004/1st January 2005 and 5th January 2005, respectively (Table 1).

Diagnosis of acute encephalitis of unknown origin in three out of the six transplant recipients initiated a second evaluation of the organ donor's medical history. During the contact investigations an acquaintance of the donor reported that she had experienced a teeth scratch by a stray dog during a trip to India in October 2004. Postexposure prophylaxis was not administered neither in India nor after the woman had returned to Germany. Thus, the strong suspicion arose that the organ donor had suffered from rabies infection and that the virus was transmitted to the recipients.

Materials and methods

Samples

Specimens were sampled from the six transplant recipients at the clinical centres and were transported to the reference laboratories. Materials were aliquoted and stored at −80 °C in freezers which were connected to a central temperature monitoring system.

Detection of rabies virus antigen by fluorescent antibody testing (FAT)

Smears of brain tissues obtained at autopsies were tested for rabies virus antigen using the standard procedure described by Dean et al. (1996). Formalin-fixed samples from the organ donor's brain were subjected to a pre-treatment with Proteinase K (Roche, Mannheim, Germany) (Warner et al., 1997).

Isolation of the rabies virus by inoculation of cell cultures (RTCIT)

Murine neuroblastoma cells (NA-C 1300, American Type Tissue Collection, Manassas, USA) were inoculated with the patients' saliva, cerebrospinal fluid, or 20%-suspensions of tissue homogenates, respectively, and subsequently incubated for 72 h. To confirm negative results in RTCIT, the cells were passaged at least three times (Müller et al., 2004; Webster et al., 1996).

Virus-typing by monoclonal antibodies

Rabies virus isolates harvested from the cell cultures were typed by using a panel of monoclonal FITC-labeled anti-nucleocapsid antibodies, as described in full details previously (Schneider, 1982).

Extraction of RNA, polymerase chain reactions, sequencing and phylogenetic analyses

Total RNA was extracted from the clinical samples by MagNa Pure LC Total Nucleic Acid Isolation Kit (Roche Diganostics, Mannheim, Germany) or RNeasy Mini Kit (Qiagen, Hilden, Germany) in accordance with the manufacturers' instructions. Highly stringent procedures as recommended by Kwok and Higuchi (1989) were applied.

For polymerase chain reactions, initially two conventional protocols with end-point detection were used: (i) A heminested assay previously designed for the detection of various lyssavirus species including classical rabies virus (RABV), Lagos bat virus (LBV), Mokola virus (MOKV), Duvenhage virus (DUVV), and the European bat lyssaviruses type 1 and 2 (EBLV-1 and 2) with primers derived from the nucleoprotein gene (PCR I). The detection limit of this PCR for genotype 1 corresponded to 0.002 tissue culture infectious doses 50 ($TCID_{50}$) (Heaton et al., 1997) and (ii) A genotype 1-specific nested PCR adapted from an already published "single round" protocol (Müller et al., 2004) (PCR II). The primers for this assay were derived from the nucleo-/phosphoprotein gene and the nucleotide sequences were as follows: N1161 (nucleotides, nts, 1161–1182, numbering according to the SAD-B19 GenBank sequence M31046, Conzelmann et al., 1990) 5′-AAGAACTTCAAGAATACGAGGC-3′ (PCR 1 and RT); N1579 (nts 1579–1560) 5′-TTCAGCCATCTCAAGATCGG-3′ (PCR 1); N1220 (nts 1220–1239) 5′-GATGGAACTGTCAACTCTGA-3′ (PCR 2) and N1539 (nts 1539–1520) 5′-CTAGGATTGACAAAGATCTT-3′ (PCR 2). By this PCR, 0.025 $TCID_{50}$ of the CVS-11 strain (American Type Tissue Collection, Manassas, USA) could be detected (Fig. 1).

Since at the beginning of 2005, real-time PCRs for the detection of lyssavirus RNA (Fischer et al., 2012) were not yet well established in the two national reference laboratories, the primary