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Fatal SV40-Associated Pneumonia and Nephropathy following Renal Allotransplantation in Rhesus Macaque

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Abstract

Recrudescence of latent and dormant viruses may lead to overwhelming viremia in immunosuppressed hosts. In immunocompromised hosts, Simian Virus 40 (SV40) reactivation is known to cause nephritis and demyelinating central nervous system disease. Here, we report SV40 viremia leading to fatal interstitial pneumonia in an immunosuppressed host following renal allotransplantation.

Keywords

SV40; opportunistic infection; renal transplant; Rhesus Macaque; viral reactivation

Introduction

Renal transplantation is the most effective treatment for End-Stage Renal Disease (ESRD) in humans. Though the associated morbidity and mortality are superior to dialysis, post-transplant outcomes remain limited by the morbidity associated with chronic non-specific immunosuppression. Therapies that permit long-term acceptance of an allograft, but with decreased rates of co-morbid side effects, are urgently needed. T-cell costimulation blockade (CoB) provides a lymphocyte-specific target for suppression of alloreactive responses to provide a platform for immunosuppression, and is now available for clinical transplantation through the CD28-B7 pathway inhibitor Belatacept. T-cell costimulation blockade permits improved renal function with decreased rates of off-target effects.

The polyomavirus subfamily (PVs) (of the Papovaviridae family) is a collection of small, non-enveloped DNA viruses. Primary infection occurs in early life and remains latent in immunocompetent hosts, but can manifest with viremia in immunocompromised and immunosuppressed conditions. Thirteen polyomaviruses have been identified in human clinical medicine [1–3]. BK virus (BKV) and JC virus (JCV) are the two most clinically-relevant members of the polyomavirus family[4–7]. Simian virus 40 (SV40) is common to the macaque, and shares many important structural and pathobiological characteristics with BKV and JCV. Primary infection with SV40 is generally asymptomatic. Chronic, dormant infection of the urogenital epithelium of the kidney, ureter and bladder may develop. Human

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tropism of SV40 is thought to have been facilitated in part as a result of widespread administration of contaminated poliovirus vaccine grown on macaque cells [7–9].

SV40-associated diseases have rarely been reported in the non-human primate (NHP) population. In primates with immune function compromised by Simian Immunodeficiency Virus (SIV), recrudescence of dormant SV40 infection causes nephritis and demyelinating central nervous system disease. In Cynomolgus, Maki et al (2008) reported loss of control of SV40 as a major limitation to post-transplant survival in a renal allotransplant model [10]. A single report describes overwhelming interstitial pneumonia in an immunocompetent *Rhesus* primate [11].

Here we report a case of severe SV40-associated pneumonia in a Rhesus Macaque following renal transplantation and costimulation-blockade-based immunosuppression.

Materials and Methods

This experiment was conducted with approval of the Duke University Institutional Animal Care and Use Committee and in adherence with the principles laid out in The Guide for the Care and Use of Laboratory Animals [12].

Rhesus macaques (*Macaca mulatta*) were obtained from breeding colonies at AlphaGenesis, Inc. (Yemassee, SC, USA). Donor-recipient pairs were determined by MHC disparity. Transplantation was performed in domino fashion to maximize utility of animals, with each animal serving as a kidney donor prior to receiving a transplant as described previously [13]. Right nephrectomy was simultaneously conducted after implantation to leave the recipient dependent on the allograft. Post-transplant monitoring consisted of daily clinical assessment by veterinary staff, as well as laboratory studies performed 2–4 times monthly. Rhesus CMV (*Cercopithecine herpesvirus 8* or Rhesus Cytomegalovirus, of the *Cytomegalovirus* genus, is an enveloped dsDNA virus. Immunosuppression increases the risk of reactivation of CMV, and as such transplant recipients receive prophylaxis and are monitored for reactivation) was monitored with every blood draw using quantitative Polymerase Chain Reaction (qPCR). CMV prophylaxis was initiated at the time of transplant, consisting of valganciclovir (an antiviral approved for prophylaxis against CMV disease in the setting of organ transplantation. Valganciclovir is a prodrug of ganciclovir) PO 60 mg twice daily. Viral copies >10,000/mL were treated with addition of ganciclovir (an antiviral approved for the treatment of CMV infection) IV 5 mg/kg twice daily.

Results

Clinical History

Left donor nephrectomy was performed, followed by renal allotransplantation three weeks later. Ketamine (an anesthetic agent) 10mg/kg IM was used for induction, and the animal was maintained on isoflurane (general anesthetic) 1–1.5% continuous inhalation. Bupivacaine (an amino-amide anesthetic) 2mg/kg was provided as local anesthetic at the incision site, and flunixin (a non-steroidal anti-inflammatory drug) 0.5mg/kg IM daily post-procedure for analgesia. Belatacept (20mg/kg IV) was given on the day of transplant, then

on postoperative days 3, 7, 14, 21, and 28, then every two weeks. Daily sirolimus (or rapamycin, an immunosuppressive agent that prevents T- and B-cell activation through inhibition of the mammalian Target Of Rapamycin [mTOR]) initiated on POD0 with initial dose of 0.05mg/kg IM, then titrated to a therapeutic range of 8–12ng/mL. A single dose of methylprednisolone (or Solu-Medrol, a corticosteroid) 15mg/kg IV was administered intraoperatively prior to graft implantation. The animal recovered uneventfully with stable renal function and hematologic profile thereafter (Figure 1). On POD63 the animal was reported for lethargy and increased respiratory rate and empiric antibiotics and IV ganciclovir were initiated. Over the following 48 hours, despite empiric therapy and supportive care, the animal failed to achieve a favorable trajectory and was humanely euthanized.

Histopathological Analysis

Liver, heart, brain, bone marrow, and allograft kidney samples were collected in 10% formalin for histological evaluation. Tissues were processed and embedded in paraffin with subsequent sectioning and hematoxylin and eosin (H&E) staining for morphologic observation. Immunohistochemical staining for SV40 was used for the diagnosis of SV40 infection in the tissues by using anti-SV40 T-antigen antibody (Abcam, Cambridge, MA), DAB labeling, and nuclei counter-staining with hematoxylin.

H&E stain of kidney tissue revealed focal mononuclear infiltration in the cortex. The overall infiltrating area occurred in less than 25% of parenchyma. Tubulitis was rare and mild and no vasculitis was identified. Prominent basophilic or amphophilic intranuclear inclusions were observed in tubular cells primarily in the medulla, the collecting ducts, distal tubules, and rarely in the cortex. Immunohistochemical staining for SV40 demonstrated strongly positive SV40 T-antigen expression in intranuclear inclusions.

Lung tissue examination showed severe vascular congestion and massive interstitial and alveolar immune cell infiltration. Enormous viral intranuclear inclusions were frequent in the lung. Immunohistochemical staining for SV40 showed that massive intranuclear inclusions were strongly positive for SV40 T-antigen. The small portions of uninvolved parenchyma with preserved alveolar structures were negative for SV40. Liver, brain, heart, and bone marrow tissue sections did not display obvious abnormality, with no intranuclear inclusions and no SV40 expression in the tissues (Fig.2).

Discussion

This is a case of fatal SV40-associated pneumonia in a Rhesus Macaque following renal allotransplantation with costimulation-blockade-based immunosuppression. Histologically, findings were notable for severe inflammation, infiltration and viral intranuclear inclusions in the interstitium and capillaries, and congestion in the vessels and alveoli. The kidney allograft showed patches of infiltration and vast intranuclear viral inclusion in tubular cells, but no evidence of rejection. Immunohistochemical staining demonstrated that intranuclear inclusions in the lung and kidney tissues were strongly positive for SV40 T-antigen, suggesting that this animal had severe SV40-associated pneumonia and nephropathy.

Though SV40 infection and SV40-driven disease have been reported in immunocompromised NHP's, this represents to our knowledge the only report of SV40-driven fatal pneumonia in an immunosuppressed Rhesus species. This presentation mirrors the findings of Maki et al (2008) in the *Cynomolgus* species, in which morbidity associated with SV40 disease limited post-transplant survival in a renal allotransplant model using a Thymoglobulin-Sirolimus-Rituximab-based immunosuppression strategy[10].

Loss of control of latent viruses and subsequent viremia is of important clinical relevance in human transplantation. In humans, JCV is the etiologic agent of demyelinating central nervous system diseases such as Progressive Multifocal Leukoencephalopathy (PML)[5–7]. Persistent infection of Polyomavirus is common, with seroprevalence estimated at 70–90% in human adults following infection in childhood [14–18]. Dormant BK virus remains confined to urogenital epithelial cells. Clinically-significant disease due to BKV infection is rare except under immunocompromised and immunosuppressed conditions [1, 3, 7]. Most commonly, disease in the renal transplant patient population manifests as polyomavirus-associated nephropathy (PVAN), which has been increasingly recognized as a significant contributor to renal allograft failure [19–22].

Experimental data generated by the Rhesus Macaque model of renal allotransplantation are highly applicable to human clinical transplantation. Though costimulation-blockade-based immunosuppression yields a superior comorbidity profile than more non-specific immunosuppression, loss of viral control, including SV40, remains an potential effect of this strategy, and has been seen in a recent CoB-sirolimus-based trial[23]. This report highlights the relevance of the Rhesus model in renal allotransplantation and demonstrates the need for close post-transplant monitoring for loss of viral control.

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Figure 1.
Longitudinal (A) serum creatinine and (B) complete blood count analysis.

Figure 2. H&E and SV40 immunohistochemistry analysis

A) H&E stain and B) SV40 stain in lung tissue. There was massive vessel congestion (A, arrow), infiltration, and intranuclear inclusions in most parts of the tissue. The SV40 stain was strongly positive in intranuclear inclusions (B, arrow). Only a small portion of tissue contained preserved alveolar structure and were negative for SV40 staining (x100, scale bar in the images represent 100 μ m. Insert in image B is higher magnification). C) H&E stain and D) SV40 stain in the kidney tissue. Many intranuclear inclusions (arrows in A and B) with strong positive SV40 expression (arrow in B) in the tubular cells are noted (x400, scale

bar in the images represent 20 μm). E) H&E stain and F) SV40 stain in the heart tissue. G) H&E stain and H) SV40 stain in the brain tissue. The morphology of heart and brain were normal without positive SV40 expression (x400, scale bar in the images represents 20 μm). Liver tissue was likewise negative for SV40 expression (data not shown).