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Activation of innate immune defense mechanisms contributes to polyomavirus BK-associated nephropathy

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Polyomavirus-associated nephropathy (PVAN) is a significant complication after kidney transplantation, often leading to premature graft loss. In order to identify antiviral responses of the renal tubular epithelium, we studied activation of the viral DNA and the double-stranded RNA (dsRNA) sensors Toll-like receptor 3 (TLR3) and retinoic acid inducible gene-I (RIG-I) in allograft biopsy samples of patients with PVAN, and in human collecting duct cells in culture after stimulation by the dsRNA mimic polyriboinosinic:polyribocytidylic acid (poly(I:C)), cytokines, or infection with BK virus. Double staining using immunofluorescence for BK virus and TLR3 showed strong signals in epithelial cells of distal cortical tubules and the collecting duct. In biopsies microdissected to isolate tubulointerstitial lesions, TLR3 but not RIG-I mRNA expression was found to be increased in PVAN. Collecting duct cells in culture expressed TLR3 intracellularly, and activation of TLR3 and RIG-I by poly(I:C) enhanced expression of cytokine, chemokine, and IFN-β mRNA. This inflammatory response could be specifically blocked by siRNA to TLR3. Finally, infection of the collecting duct cells with BK virus enhanced the expression of cytokines and chemokines. This led to an efficient antiviral immune response with TLR3 and RIG-I upregulation without activation of IL-1β or components of the inflammasome pathway. Thus, PVAN activation of innate immune defense mechanisms through TLR3 is involved in the antiviral and anti-inflammatory response leading to the expression of proinflammatory cytokines and chemokines.

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Polyomavirus-associated nephropathy (PVAN) is an emerging cause of significant kidney transplant dysfunction affecting up to 10% of organ recipients, often leading to graft loss. Most cases of PVAN are elicited by BK virus (BKV) in the context of intense immunosuppression. As no specific antiviral drug is available to date, the primary treatment for PVAN is reduction of immunosuppression, which must be carefully balanced against increased risks of acute rejection (AR).[1,2]

BKV normally replicates in urothelial cells, which remains asymptomatic in two-thirds of infected patients after kidney transplantation,[3] but it can exhibit tropism for the renal tubular epithelium, which may be latently infected.[4]

The renal involvement in PVAN is multifocal, and distal nephron segments, that is, medullary collecting ducts and distal cortical tubules, are usually more severely affected than proximal segments.[5] To achieve a productive infection, the BKV genome has to be delivered to the nucleus, where early genes are expressed, followed by DNA replication, late protein expression, and virion assembly. Histologically, viral replication results in tubular epithelial cell enlargement, karyomegaly, and nuclear inclusion bodies. These cytopathic changes are often associated with death of tubular epithelial cells, denudation of the basement membrane, and a strong interstitial inflammatory response, similar to that seen in acute interstitial rejection. Histological diagnosis of PVAN requires evaluation of a renal biopsy with demonstration of cytopathic changes and confirmation by immunohistochemistry.[6]

The molecular events involved in BKV invasion of host cells and subsequent intracellular trafficking are an important area of study. In various cell culture models, BKV uses an N-linked glycoprotein containing an α(2,3)-linked sialic acid as a receptor and enters cells through caveola-mediated endocytosis.[7,8] After this relatively slow and cholesterol-dependent internalization, BKV migrates through the cytoplasm and via cellular components to the nucleus, where viral transcription, replication, and assembly take place. The

innate immune system has a critical role in recognizing viral infections and evoking initial antiviral responses. Viruses are sensed via their nucleic acid genome or as a result of their replicative or transcriptional activity.[9] Several classes of receptors sense cytosolic viral components, such as double-stranded RNA (dsRNA), single-stranded RNA (ssRNA), and ds or ss DNA. These include retinoic acid-inducible gene I (RIG-I)-like receptors, Toll-like receptors (TLRs), nucleotide oligomerization domain-like receptors, and cytosolic DNA sensors.[10,11] Recognition by these sensors induces the production of type I interferons (IFNs) and the assembly of inflammasome complexes that activate caspase-1, leading to production of interleukin-1β (IL-1β) and IL-18.[12,13]

For polyomavirus BK, dsDNA and dsRNA are critical targets. The dsRNA is a molecular pattern associated with viral infection, because it is produced by most viruses at some point during their replication.[14] The recognition of viral dsRNA triggers downstream signaling cascades leading to the activation of nuclear factor (NF)-κB, and induces antiviral mediators such as type I IFNs and proinflammatory cytokines.[15,16] RIG-I is a highly inducible cytoplasmic RNA helicase that signals antiviral responses after binding dsRNA and ssRNA containing a 5′ triphosphate. This pathway has been implicated in antiviral responses to Sendai virus, vesicular stomatitis virus, Newcastle disease virus, as well as different flaviviruses and Kaposi's sarcoma-associated herpesvirus.[17–19] In addition, Chiu *et al.*[20] recently demonstrated that a cytosolic B form of dsDNA, poly (dA-dT) · poly (dA-dT), is able to activate RIG-I and induce IFN-β production via the cytosolic DNA-dependent RNA polymerase III (PolIII).

Because of the above-mentioned characteristics involved in BKV invasion of host cells and subsequent intracellular trafficking, we restricted our search for TLR candidates that could be involved in PVAN to those intracellular TLRs that sense nucleic acids in endosomal compartments. This latter group includes TLR3, TLR7, TLR8, and TLR9. Whereas TLR7 and TLR8 recognize ssRNA and TLR9 specifically binds CpG DNA motifs and has a significant role in the development of tubulointerstitial injury in systemic lupus,[21] TLR3 recognizes viral dsRNA, which is generated during the life cycle of many viruses, and its synthetic analog polyriboinosinic:polyribocytidylic acid (poly(I:C)).[22,23] TLR3 senses dsRNA through its unique adaptor protein TIR-domain-containing adapter-inducing interferon-β (TRIF).[24] Recognition of viral RNA by TLR3 triggers activation of the transcription factors NF-κB and interferon regulatory factor 3 and induction of type I IFNs, which are critical for cellular antiviral responses.

We hypothesized that particularly TLR3 could be a receptor candidate to mediate activation of innate immunity in PVAN. Our hypothesis was underscored by two recent findings. (1) TLR3 resides in the endosomal membrane and the endoplasmic reticulum and moves to dsRNA-containing endosomes in response to dsRNA.[25] As BKV has previously been shown to enter cells through endocytosis, viral dsRNAs could activate TLR3 during BKV infection of tubular epithelial cells upon viral entry and uncoating in the endosome. (2) Upregulation of the TLR3 pathway was reported in response to various viruses containing a dsDNA genome, for example, Kaposi's sarcoma-associated herpesvirus,[26] HSV-1,[27,28] and murine CMV.[29]

Here we examined the activation of TLR3 and RIG-I in allograft biopsy samples of PVAN and in human collecting duct cells after poly(I:C) and cytokine stimulation, as well as after infection with BKV. Our findings indicate that activation of innate immune defense mechanisms, that is, TLR3, contributes to the inflammation in PVAN.

RESULTS

In biopsies with PVAN staining for polyomavirus (anti-SV40) and TLR3 colocalized in epithelial cells of cortical tubules and the collecting duct

Polyomavirus BK exhibits a tropism for the renal tubular epithelium, particularly in distal nephron segments. Immunofluorescence double staining (Figure 1) for the SV40 large T antigen (T-Ag) of polyomavirus and TLR3 in allograft biopsies with histological diagnosis of PVAN showed

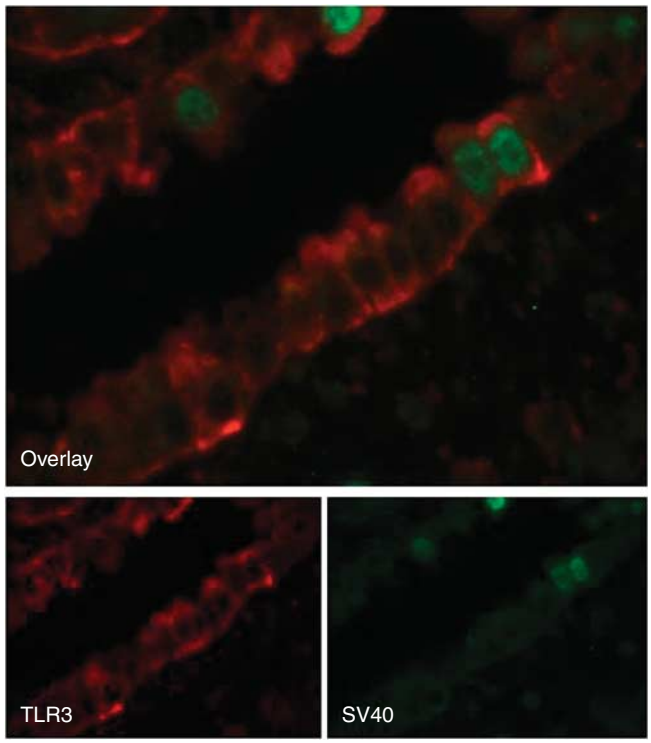

Figure 1 | Immunofluorescence double staining for Toll-like receptor 3 (TLR3) and SV40 in human kidney biopsies with clinical and histological diagnosis of polyomavirus-associated nephropathy (PVAN): colocalization of polyomavirus (anti-SV40) and TLR3 protein expression in epithelial cells of distal cortical tubules and the collecting duct. Double immunofluorescence staining of paraffin-embedded tissue sections of kidney needle biopsies from individuals with PVAN. Colocalization of nuclear signals for polyomavirus (SV40, green) and intracytoplasmatic signals for TLR3 (red) in tubular epithelial cells of distal cortical tubules and the collecting duct.