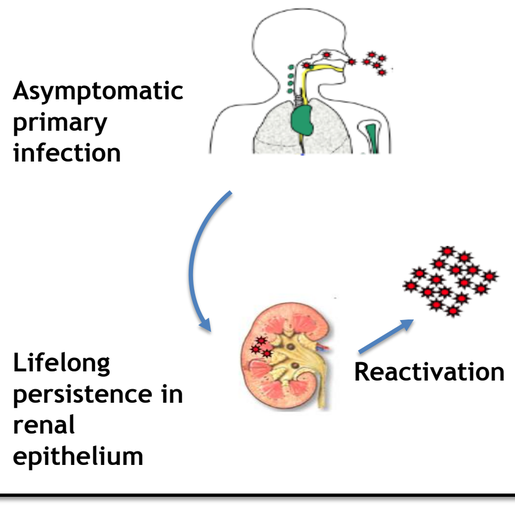
Neutralization escape in kidney transplant recipients with persistent BK polyomavirus replication

D. McILROY[1,2, 3], M. HOHNEMANN[4], Y. VERRES[1,2], C.PELTIER[1,2], F. HALARY[1,2], A. RODALLEC[5], E.PRZYROWSKI[5], M. HOURMANT[2,6], C.BRESSOLLETTE-BODIN [1,2,5,7]

1. CRTI, UMR 1064, INSERM, Université de Nantes; 2. ITUN, CHU Nantes; 3. Faculté des Sciences et des Techniques, Université de Nantes; 4. Institut für Virologie, Universität Leipzig; 5. Service de Virologie, CHU Nantes; 6. Service de Néphrologie et Immunologie Clinique, CHU Nantes; 7. Faculté de Médecine, Université de Nantes

1. Introduction

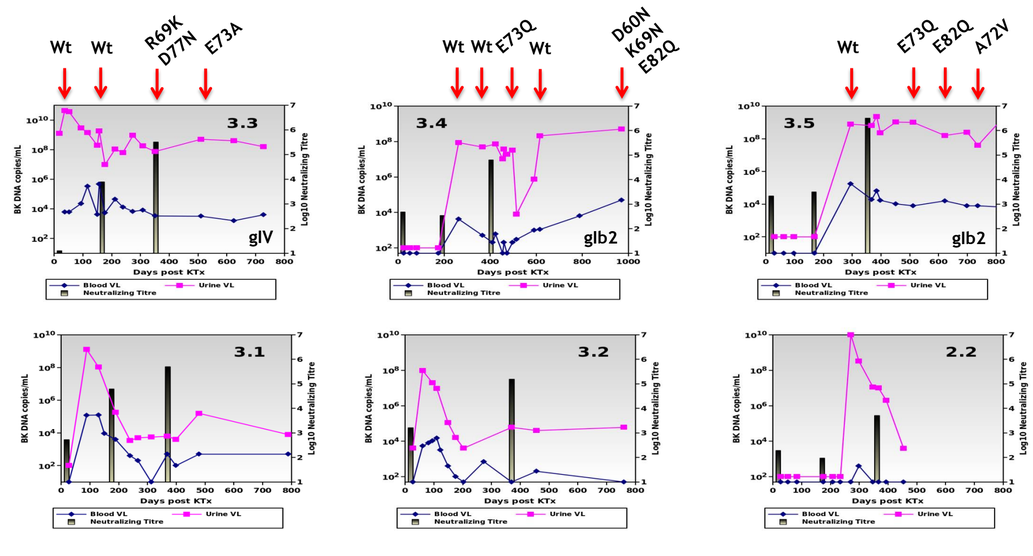
The BK polyomavirus (BKPyV) infects more than 80% of the adult population in Europe. The virus persists in a latent state in the kidney and active replication is only seen in immunosuppressed individuals, such as kidney transplant (KTx) recipients, in whom BKPyV reactivation is associated with interstitial nephropathy and allograft dysfunction.



No specific antiviral therapy

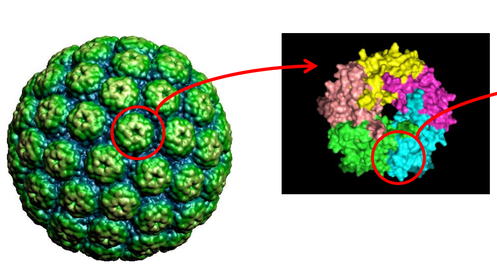
2. VP1 gene evolution in KTx patients

Non-synonymous VP1 mutations accumulate over time in patients with persistent viremia despite the presence of a robust humoral response

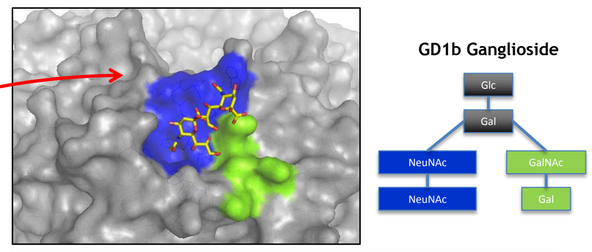


No VP1 mutations found by Sanger sequencing in urine of patients who controlled BKPyV reactivation

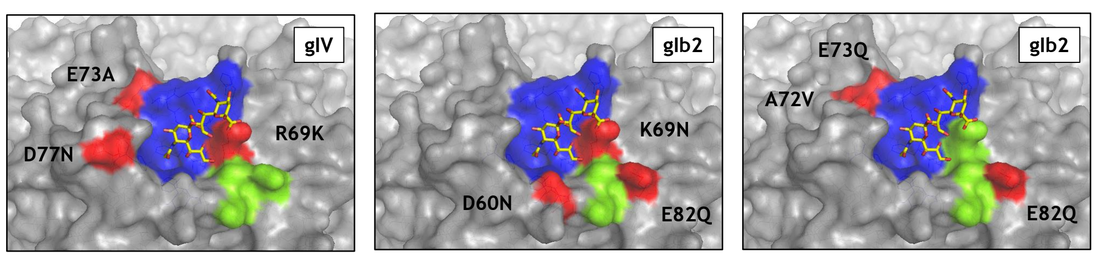
3. Location of VP1 mutations on BKPyV capsid



Capsid consists of 72 VP1 Pentamers



Yellow : α2-8 disialic acid
Blue : VP1 AA interacting with disialic acid
Green : VP1 AA interacting with Gal-GalNAc (predicted)



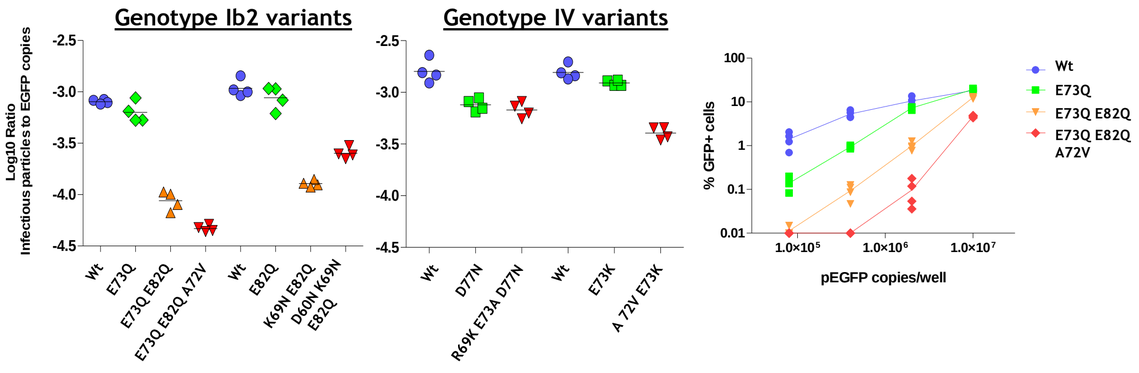
VP1 mutations cluster on the external surface of the capsid proximal to the receptor binding site

4. Effects of VP1 mutations on infectivity

Pseudotype production of different VP1 variants:

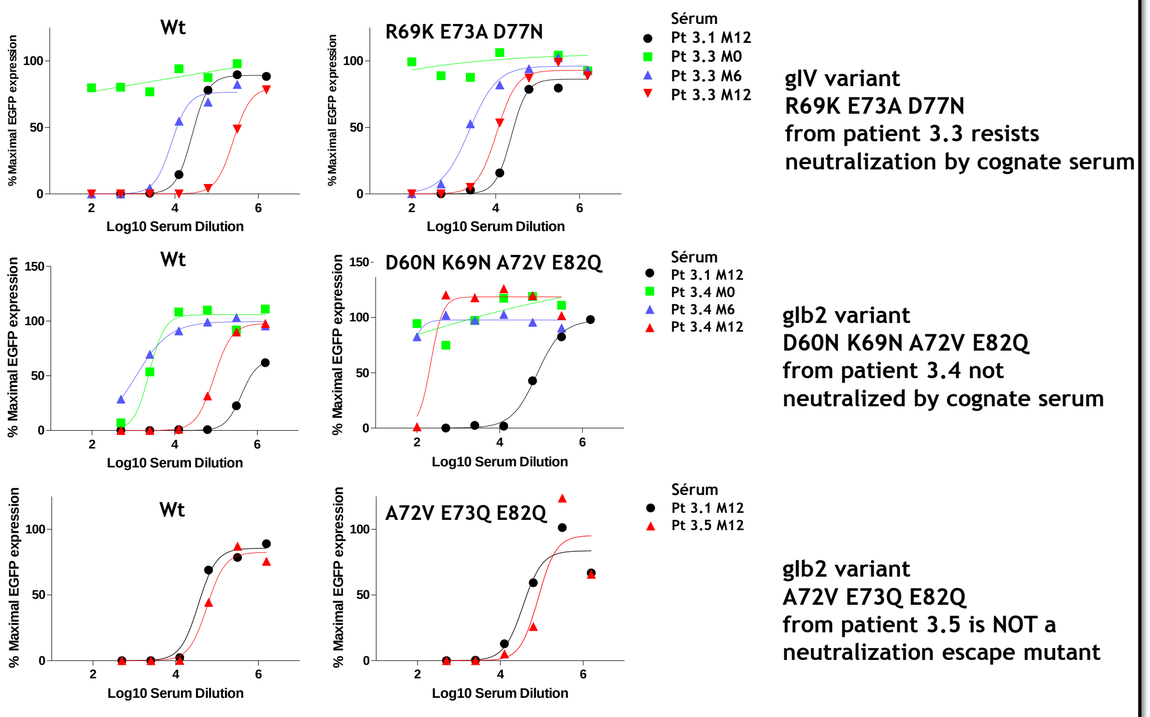
1. Mutagenesis to generate panel of VP1 variants
2. Co-transfect into 293TT cells with VP2, VP3 and EGFP
3. Extract and purify on Optiprep gradient
4. Quantify by qPCR - number of pEGFP copies/µL
5. Test pseudotype infectivity in 293TT cells

Name	Time	Sequence
AEE	M9	^{71}SAENDFSSDSPERKM
AQE	M17	^{71}SAQNDFSSDSPERKM
AQQ	M21	^{71}SAQNDFSSDSPQRKM
VQQ	M24	^{71}SVQNDFSSDSPQRKM



VP1 mutations reduce the ability of genotype I and genotype IV capsid proteins to mediate entry into 293TT cells

5. VP1 mutations and neutralization escape



glV variant
R69K E73A D77N
from patient 3.3 resists neutralization by cognate serum

glb2 variant
D60N K69N A72V E82Q
from patient 3.4 not neutralized by cognate serum

glb2 variant
A72V E73Q E82Q
from patient 3.5 is NOT a neutralization escape mutant

6. Conclusions

Evolution of VP1 is consistently observed in KTx recipients with sustained viremia and viruria. Non-synonymous VP1 mutations **reduce** the efficacy of entry into 293TT cells mediated by both genotype I and genotype IV VP1 proteins.

In addition, clear neutralization escape from cognate serum was observed in one patient with glb2 infection (3.4), and one patient with glV infection (3.3). Both of these variants were neutralized as effectively as wild-type VP1 by serum from patient 3.1 who had successfully resolved post-KTx BKPyV viremia. These results indicate that the humoral response against BKPyV in KTx recipients is heterogeneous, since some patients develop a narrow neutralizing response that is vulnerable to neutralization escape mutants. They suggest that neutralization escape contributes to the persistence of BKPyV replication in patients with durably high viral loads.

Nevertheless data from patient 3.5 indicate that lower infectivity in 293TT cells is not always accompanied by neutralization escape. One possible explanation is that the VP1 variant observed in this patient was associated with a modification of viral tropism.