

OTKA azonosító: 73145

Típus: K

Vezető kutató: Gergely Lajos

Human herpesvirus 6 (HHV-6), mostly variant B reactivation in renal transplant patients has been published previously, but the pathogenetic role of HHV-6 variant A has not been clarified. During the project blood samples from renal transplant patients were collected to determine the prevalence of HHV-6 variants, and to investigate the interaction between HHV-6 viraemia, human cytomegalovirus (HCMV) infection and clinical symptoms. In order to examine the virus nested PCR and to quantify the viral load, real-time PCR were used. Since the virus is able to establish latency, beside the quantitative examination, RT-PCR was used to prove the active viral replication. Human cytomegalovirus infection was detected by pp65 antigenaemia test. During the study 200 renal transplant and 200 healthy blood donors were studied. Active HHV-6 infection, viraemia was significantly more frequent in renal transplant patients than in healthy individuals, at the same time the frequency of latent infection did not show difference. The viral infection did not show any correlation with the time after transplantation, virus replication was detected also early and late after transplantation. Latent infection was caused mainly by the B variant HHV-6 in both studied groups. An interesting data of the project was that contrary to previous publications, HHV-6 viraemia in renal transplant patients was caused dominantly by HHV-6 A variant. Statistically we did not find interaction between HHV-6A infection and the clinical symptoms of the patients. HCMV reactivation was detected also in renal transplant patients, the frequency was lower than that of the HHV-6 infection. Simultaneous presence of HCMV and HHV-6 infection was not detected. There were no significant differences between the age of HCMV positive and negative patients, between the time of sample taking after the transplantation of HCMV positive and negative patients. The final result of this project was that we establish good diagnostic procedures for HHV-6 infections of renal transplant patients which nowadays is thought to be as important as the HCMV infection. Our study also strengthen the suggestion that diagnosis of HHV-6 infection in renal transplant patients is required. Although the clinical significance of HHC-6A infection is not clarified, our data provided new, important information to this research filed: dominance of A variant infection was revealed in renal transplant patients, since previously the B variant was published as frequent pathogen. Data were published in international journal.

Owing to the more potent immunosuppressive treatment of renal transplant patients, improved survival of grafts in organ transplant patients can be achieved, but risk for opportunistic infections, reactivations of latent infections and reinfections are increasing. In renal transplant patients loss of functional antiviral immunity can result in lytic infections caused by a human pathogen polyomavirus, namely BK virus (BKPyV) with severe

consequences. BKPyV is associated with ureteral stenosis, hemorrhagic cystitis and polyomavirus nephropathy (PVN) mainly in renal transplant patients. Viruria occurs first within 2-6 months of kidney transplantation which can be followed by viraemia after some weeks later. BKPyV replication affects approximately 80 % of renal transplant patients. PVN develops in 10 % of them resulting in allograft failure in 50-90 %, and half of these cases lead to graft loss mostly after 1 year of transplantation, when the degree of immunosuppression by drugs facilitates this process. Screening of renal transplant patients for BKV viraemia and viruria is highly recommended regularly during the first two years of posttransplantation. In 2007 two new human polyomaviruses, WU and KI (WUPyV, KIPyV), then later up to now 9 other, new polyomaviruses were discovered. Since in our project samples from renal transplant patients were collected, we decided to screen these new viruses also in the samples. The other explanation is that we did not follow the in vitro study, since the budget was restricted and without further grant it was not possible to complete. As a result of this we expanded the project with new viruses, more samples from patients. Our aim were to examine the prevalence of new human polyomaviruses, namely KIPyV, WUPyV, HPyV9 and BKPyV, the possible interaction between them and their clinical significance. The information at the time of the beginning was limited. Analogously to BKPyV it was hypothesized that these new viruses may have clinical importance in immunocompromised, for example transplant patients. The way of transmission, the time of primary infection, the symptoms of primary infections, the clinical consequences was not known. We performed a study to examine the prevalence of newly described human polyomaviruses in different samples. We found KIPyV, WUPyV DNA in respiratory (10 %), blood (3.6 %) and urine samples (14 %) of renal transplant patients mainly early after the transplantation, but not in samples from healthy blood donors. These data were published in international journal. We found and published first the presence of these viruses in urine sample and discussed that possibly these viruses may infect also the kidney, urinary tract similarly to BKPyV. These data and some previous publications about the higher frequency of these viruses in immunocompromised patients suggest that KIPyV and WUPyV might have clinical importance in these patients, and urine samples can be also the source for infections. Consequently, about a year ago we started a follow up study with renal transplant patients to examine that after the transplantation what is the frequency of HPyV9, WU and KI viruses, what the clinical consequences are, whether these viruses are able to establish latency, reactivations are possible. Today we have new data under publication about the HPyV9 virus. The OTKA project number will be referred in this

article also. From the collected samples we have valuable prevalence data, sequence information which will be published (if the study is completed) in the near future.

Transient immunosuppression because of pregnancy may lead to reactivation of BKPyV resulting in generally asymptomatic viruria and viraemia. It was also hypothesized that the new human polyomaviruses may infect pregnant women more frequently or reactivation due to the immunosuppression may result in reactivations. In our study significantly higher prevalence of BK viruria was observed in pregnant women compared with non pregnant women. Human polyomavirus 9 was found in plasma, urine and respiratory samples from pregnant women but not more frequently than in samples from non pregnant women. Our work-team published first the presence of HPyV9 in respiratory samples suggesting that respiratory transmission of this virus may be possible. WU and KI viruses were not detected in any of the studied samples from pregnant women. These data were published in international journal.

Our work-team joined to a new research field to investigate the pathogenetic role of the newly described human polyomaviruses. The clinical importance and many other important questions are not clarified today, but we found and published new, valuable data, hypotheses about these viruses. Three articles are published and two others are under publications. We started a new research with a hot topic in virology which serves a basis for further study.

Five sequential human papillomavirus type 11 (HPV11) positive samples collected from an aggressive juvenile onset recurrent respiratory papillomatosis before, during and after intralesional cidofovir therapy leading to virological failure after initial response were analyzed. Sequencing of the complete genome as well as methylation analysis by bisulfate modification and sequencing of the long control region (LCR) were performed to seek for genetic and epigenetic changes as a possible background for therapy failure. Single-strand conformation polymorphism of E1, E2, E6, E7 and LCR was used to exclude the presence of multiple HPV11 infection. All five complete genomes were identical and all four E2 binding sites in the LCR were uniformly unmethylated in all five genomes. Thus the virological failure was not due to virological factors suggesting that cidofovir action may depend more heavily on the host.

The second part of the study compared complete genome sequences of human papillomavirus (HPV) type 11 from two solitary papillomas (considered minimally aggressive), two

moderately (six and nine episodes) and two highly aggressive (30 and 33 episodes) juvenile-onset respiratory papillomatosis.

Genomic regions were sequenced using the Sanger method; sequences were compared to available GenBank genomes. Activity of the long control region (LCR) was assessed in HEP-2 cell line using luciferase assays and compared to that of the reference (GenBank Accession No.: M14119). Site-directed mutagenesis was performed to confirm the association of polymorphisms with differences in LCR activity.

Thirteen alterations resulted in twelve amino acid changes in different open reading frames (ORFs). A72E in E1 and Q86K in E2 proteins were exclusively present in a moderately aggressive disease, L1 alterations A476V and S486F were unique to a severe papillomatosis. HPV11s in both solitary papillomas had identical LCRs containing a T7546C polymorphism, which strongly attenuated LCR activity, as confirmed by site-directed mutagenesis. All other sequences showed significantly higher activities which corresponded well to severity, excepting the highly aggressive papillomatosis with the L1 alterations. High LCR activities were associated with a deletion at position 7509 or a T7904A polymorphism (next to an E2-binding site); the role of both were also shown in mutant LCRs generated by site-directed mutagenesis.

Presence of intratypic variants cannot explain differences in severity of HPV11-associated respiratory papillomatosis; alterations in the L1 ORF and the LCR are likely to play a role in the severity of the disease.

Diversity of TTV1 was assessed in the head and neck region in patients with potentially malignant (oral lichen planus, oral leukoplakia) and malignant lesions (oral and laryngeal squamous cell cancers) and was compared to that found in the uterine cervix (cervical atypia and cervical cancer) by directly sequencing the NG061-063 segment of ORF1. These sequences were classified by the formerly used genogroup-genotype system as well as by the newly accepted species classification by aligning with the corresponding region of the type sequences of the 29 TTV species. All sequences obtained during the study clustered together with the TTV1 type sequence; to express diversity within TTV1, genotypes and subtypes of the former classification were used.

The commonest subtypes were 2c followed by 2b, 1a and 1b. Subtypes 2b and 2c were evenly distributed among cervical samples; subtype 1a was more frequent in patients with cervical

atypia or cancer. Subtypes 2c was more frequent than 2b in head and neck lesions. In conclusion, genotype and even subtype distribution may be important in association with diseases, therefore using this classification for characterization of intraspecies diversity of TTV1 is proposed.