

## VIRUS ISOLATION FROM THE KIDNEY, TUMORS AND LYMPH NODES OF PATIENTS WITH BEN

B. Uzelac-Keserović<sup>1</sup>, K. Apostolov<sup>2</sup>

<sup>1</sup>Institute of Immunobiology and Virology, Torlak, Belgrade, Serbia

<sup>2</sup>Biology Section, Macedonian Academy of Arts and Science (MANU), Skopje, Macedonia

### Introduction

This paper is based on two recent publications (1,2), and one submitted for publication (submitted for publication). There is an important correction. In the two previous publications in *Nephron* it was stated that the virus isolated from the kidneys, the tumors and metastatic lymph glands was classified as a coronavirus (1,2). Subsequent more detailed investigations revealed that the virus should not be identified as a coronavirus (submitted for publication). However, the evidence presented in this work suggests strongly that the newly isolated virus is involved in the etiology of BEN. The new findings open the possibility of reassessment of this still mysterious disease.

### Virus isolation

The virus can be readily isolated from tissue cultures of freshly collected tissues obtained at operations for urinary tract tumors. In this respect we are indebted to the enthusiastic involvement of Dr. Vasic from the Urology Department of the District Hospital in Doboj in Bosnia and Herzegovina. The primary tissue cultures were prepared by the approved methods (1,2). In the primary cultures there was no obvious cytopathic effect (CPE). However, when the supernatant was inoculated in VERO cells a clear CPE was obtained. The same was obtained on the co-cultivation of the primary cultures with VERO cells. The titration of the CPE came to  $10^{-6}$  (1,2)

### Virus identification

Efforts to identify the virus based on electron microscopy and immunofluorescence led to the tentative

conclusion that it was a coronavirus. However, it is clear that it is a new virus as yet unclassified (Riquelme C, Escors D, Ortego J, Sanchez CM, submitted for publication).

### Serology

The isolated virus was used to raise antibodies in rabbits. The neutralising antibodies titre of the serum obtained was 1/1024. The titration of neutralising antibody of patients sera using the virus isolated was conducted by standard methods.

### Results

The operations indicated for removal of the tumors associated with BEN provide fresh tissue for establishing primary cultures. The virus is easily demonstrated in the widely available VERO cells, a cell line established from monkey kidney cells. There was a clear CPE and the titre is  $10^{-6}$ . The virus was isolated from the patients kidney tissue, from the tumors as well as from the metastatic lymph nodes. The titre of the virus was the same at  $10^{-6}$ , for all the isolates and cross-neutralization investigation indicated a single strain.

The initial results of serological surveys using neutralising and immunofluorescent antibodies has been presented. In this work we present, in our opinion the more relevant results, on patients on dialysis with and without BEN and patients affected with tumors. It may be seen that 57/65 of BEN patients on dialysis were positive, and only 2/12 of patients on dialysis no BEN were positive in low titres. In urotelial tumors all the 13/13 of BEN patients were positive.

Table 1. Neutralising Antibody Titres in Patients Sera

Patients	No.	1/16	1/32	1/64	1/126	1/256	1/512	Total +/	%
BEN on dialysis	65	12	6	12	18	7	2	57	87.7
On dialysis no BEN	12	2	0	0	0	0	0	2	16.6
Tu and BEN	13	0	0	1	3	4	5	13	100
Tu non BEN	13	0	1	0	0	0	0	1	7.7

## Discussion

A new virus was isolated from cases of BEN patients with tumors. The virus was isolated from the kidneys, from the tumors and significantly from the metastatic lymph glands. These results, supported by serology, support strongly a virus aetiology for this disease.

Some epidemiological data, as discussed previously, indicate that it is likely the virus has its origins in a zoonosis and that the humans are an incidental host (1,2).

However, many questions remain unanswered. A list would include: identity of the virus, further serology, the mechanism of viral persistence, animal host, the cause of the tumors, the feasibility of a vaccine, antiviral chemotherapy. The virus itself is available to laboratories from B.U.K.

## References

1. Apostolov K, Spasić P, Bojanić N. Evidence of a viral aetiology in endemic (Balkan) nephropathy. *Lancet* 1975; ii: 1271-1273.
2. Uzelac-Keserović B, Spasić P, Bojanić N, et al. Isolation of a coronavirus from kidney biopsies of Endemic Balkan Nephropathy patients. *Nehron* 1999; 81: 141-145
3. Uzelac-Keserovic B, Vasic D, Ikonovovski J, Bojanic N, Apostolov K. Isolation of a coronavirus from urinary tract tumours of endemic Balkan nephropathy patients. *Nephron* 2000; 86(1): 93-94.

## Conclusions

A virus was isolated from kidneys, tumors and metastatic lymph glands from cases of BEN. The material was obtained at operations of tumors of the urinary tract. The identity of the virus has yet to be established. In cases of BEN with tumors, antibodies were found in *all* the patients. The identity of the virus is the subject of a companion paper (Riquelme C, Escors D, Ortego J, Sanchez CM, Uzelac-Keserovic B, Enjuanes L. submitted for publication). The evidence presented suggests strongly that as yet an unidentified virus is involved in the aetiology of BEN. There are still many unresolved questions concerning the putative virus.