Acta virol. **31** : 180—184, 1987

PARTIAL CHARACTERIZATION OF A HANTAVIRUS ISOLATED FROM A *CLETHRIONOMYS GLAREOLUS* CAPTURED IN BELGIUM

[1]G. VAN DER GROEN, G. BEELAERT, G. HOOFD, *H. LEIRS, *R. VERHAGEN, **H. YAMANISHI, ***E. A. TKACHENKO, ***A. P. IVANOV

Institute of Tropical Medicine, Nationalestraat 155, 2000 Antwerpen, Belgium; *Laboratory of General Zoology, State University Centre Antwerp, Belgium; **Research Institute for Microbial Disease, Osaka University, Japan; and ***Institute of Poliomyelitis and Viral Encephalitides, Academy of Medical Sciences of the U.S.S.R., Moscow, U.S.S.R.

Received April 23, 1986

Summary. — A Hantavirus was isolated in Vero-E6 cells from lungs of a free living bank vole (*Clethrionomys glareolus*) captured in Turnhout, Province of Antwerp — Northern part of Belgium. With help of monoclonal antibodies the Belgian Hantavirus isolate could be clearly differentiated from Hantaan virus strain 76—118, Prospect Hill virus strain PH1 and SR11, a Hantavirus isolated from laboratory Wistar rat in Japan, but not from the nephropathia epidemica virus strain Hällnäs.

Key words: Hantavirus, Vero-E6 cells; free living bank vole; monoclonal antibodies; Hantavirus nephropathy

Introduction

Using the indirect immunofluorescent antibody technique we have demonstrated antibodies against Hantaan virus in Belgian sera from humans, wild and laboratory rodents (van Ypersele de Strihou *et al.*, 1983; van der Groen *et al.*, 1983; Desmyter *et al.*, 1983). Previously we also reported 43 Belgian and French cases with a mild form of Hantavirus nephropathy (van Ypersele de Strihou *et al.*, 1985). We now present further evidence for the existence of a Hantavirus (Desmyter *et al.*, 1985) in Belgium by virus isolation from lungs of wild *Clethrionomys glareolus* in the E6 clone of Vero cells, a continous African green monkey kidney cell line.

Material and Methods

Clethrionomys glareolus trapped on a study area in Turnhout, Province of Antwerp, Northern part of Belgium, during November 1984, were tested by an ELISA capturing technique on the presence of Hantaviral antigen (Daneš *et al.*, 1986).

[1]Requests for reprints should be addressed to Dr. Sc. Guido van der Groen, Institute of Tropical Medicine, 155 Nationalestraat, B-2000 Antwerpen, Belgium.

Table 1. Indirect immunofluorescent antibody titres to different Hantaviral antigens[b] in monoclonal mouse ascitic fluids and sera from patients and animals with Hantavirus infection

Origin of serum		Immunofluorescent antibody titre [a]				
		CG 13891	NE	PH	HNT	SR 11
Belgium						
— convalescent	V84—66	512	512	64	128	256
	V84—58	1 024	512	256	128	256
— lab personnel [c]						
	BF 17/2	—(g)	—	16	128	64
	VB 8/4	16	32	32	256	256
— lab rat [c]						
	13	128	256	64	2 048	2 048
	22	32	128	32	2 048	2 048
Finland TM 2560 [d]		8 192	16 384	16 384	4 096	8 192
France V84—47		1 024	1 024	1 024	128	256
USSR West		1 024	2 048	1 024	1 024	1 024
USSR Far East		64	128	256	4 096	4 096
— monkey NE [e]		512	1 024	1 024	256	256
— monkey PH		256	512	2048	64	128
— mouse monoclonal [f]						
	43 B	2 048	1 024	4 096	1 024	1 024
	133 E	2 048	4 096	2 048	4 096	4 096
	141 D	1 024	1 024	—	2 048	1 024
	19 B	—	—	—	65 536	—
	B 8	—	—	—	—	4 096
	B 61	—	—	—	—	16 384

Lung suspensions of 6 antigen and 6 antigen positive animals were mixed with fresh Vero-E6 cells and maintained in growth medium Rega 3 (Gibco) with 5 % heat inactivated foetal calf serum and antibiotics.

Seven days post-inoculation (p.i.) growth medium was refreshed. Fifteen days p.i. the cells were refreshed with maintenance medium (growth medium with 2 % foetal calf serum) and this was repeated at weekly intervals. The first passage was done 4 weeks p.i. and subsequently every two weeks. At each passage cells were examined for Hantavirus antigen by the indirect immunofluorescent antibody technique (van der Groen *et al.*, 1985).

Results

Intracytoplasmic, dotted form of fluorescence first appeared in inoculated E6 cells on the third blind passage, approximately 40 days after primary co-cultivation of infected lung cells with E6 cells. From the 5th passage on virtually 100 % of cells exhibited specific viral fluorescence. Out of 12 *Clethrionomys glareolus* tested one Hantavirus (strain CG 13891) has been isolated.

Legend te Table 1 (continued)

(a) Titres expressed as reciprocal of highest serum dilution giving specific fluorescence.
(b) Acetone fixed and gamma irradiated Vero E6 cells infected with the following Hantaviruses were used:

CG 13891: from *Clethrionomys glareolus* captured in Turnhout, Northern part of Belgium, 4th passage in Vero E6 cells.
— NE: Hällnäs strain, isolated from *Clethrionomys glareolus* in Sweden, lot no 568/5 and 21/2/85.
— PH: Prospect Hill-PH1 strain isolated from *Microtus pennsylvanicus* in USA, lot no 569/7 and 21/2/85.
— HNT: Hantaan strain 76-118 isolated from *Apodemus agrarius* in Korea, lot no 547 and 21/2/85.
— SR11: Isolated from a laboratory Wistar rat in Japan, lot no 570/7 and 21/2/85.

(c) Sera from personnel and rats involved in a laboratory outbreak of Hantavirus disease as described previously (Desmyter *et al.*, 1983).
(d) Convalescent phase sera from patients with a mild form of Hantavirus disease in Finland, France and the Western part of the U.S.S.R. The serum from the Far-Eastern part of the U.S.S.R belongs to a patient with a more severe form of Hantavirus disease.
(e) Anti-Hällnäs NE (B1) C119 monkey 3. 21. 84 serum and anti-PH1 PHV C115 monkey 2. 27. 84 serum as well the virus strains PH1, NE Hällnäs and SR 11 were kindly provided by Dr. D. Goldgaber.
(f) Monoclonals 43B, 133E, 141D, 19B are mouse ascitic fluids obtained from BALB/c mice inoculated with Hantaan virus strain 76—118 nad B8, B61 are monoclonals obtained after inoculation with Hantavirus strain B1, isolated from a rat in Japan as described previously (Yamanishi *et al.*, 1984).
(g) Negative at a dilution 1 : 16.

The pattern of fluorescence was similar to that seen in E6 cells infected with other Hantaviruses. No cytopathic effect has been observed. No specific fluorescence was observed with control human sera or with a group specific mouse monoclonal antibody against Reoviruses (kindly forwarded by Dr. W. K. Joklik). Specific staining of the Belgian Hantavirus strain CG 13891 was observed with convalescent sera of patients with a mild form of haemorrhagic fever with renal syndrome (HFRS), reference sera made in monkeys against Prospect Hill and Nephropathia Epidemica (strain Hällnäs), and with monoclonal antibodies prepared against Hantaan virus (Table 1).

The Belgian Hantavirus strain CG 13891 and NE virus (strain Hällnäs) react equally well with convalescent phase sera of patients with a mild form of Hantavirus disease in Belgium, Finland, France and the Western part of the USSR. However, these sera showed a lower titre on Hantaan and SR-11 virus infected E6 cells. The convalescent phase serum of a patient with a more severe form of Hantavirus disease (USSR Far East serum, see Table) reacted less well with the CG 13891 and the Hällnäs strain. The affinity of sera from Belgian laboratory personnel and rats for the Belgian Hantavirus CG 13891 was 16—64 times lower than for Hantaan and SR-11, the latter being a strain isolated from a laboratory Wistar rat in Japan. The monoclonals were able to differentiate the Belgian CG 13891 strain from PH, HNT and the SR-11 strain, but not from NE (Hällnäs).

Discussion

Present data suggest that the Belgian Hantavirus strain CG 13891 and the Hällnäs strain were closely related and were different from the Far-Eastern Asiatic strains. These data also indicated that the laboratory outbreak in Belgium was caused by a virus different from the one circulating in the rest of the population. The close antigenic relationship between CG 13891 and the Hällnäs strain corresponded well with the mild form of the Hantavirus disease previously reported in Belgium (van Ypersele de Strihou *et al.*, 1985).

Studies are underway to determine the possible aetiological role of CG 13891 in patients with a mild form of Hantavirus disease in Belgium.

These data strengthen the belief that bank vole is the main reservoir of Hantaviruses in Europe (Niklasson *et al.*, 1984; Brummer-Korvenkontio *et al.*, 1982; Daneš *et al.*, 1986; Tkachenko *et al.*, 1983; Grešíková *et al.*, 1986; Gavrilovskaya *et al.*, 1983).

Acknowledgement. — This work was performed with a grant No. 3. 0082. 86 of Fonds voor Geneeskundig Wetenschappelijk Onderzoek.

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