Acta virol. 31 : 499—503, 1987

CHARACTERIZATION OF HANTAVIRUSES USING MONOCLONAL ANTIBODIES

G. VAN DER GROEN, K. YAMANISHI+, J. MCCORMICK++, G. LLOYD+++, E. A. TKACHENKO++++

Department of Microbiology, Institute of Tropical Medicine, Antwerp, Belgium, +Department of Virology, Research Institute for Microbial Diseases, Osaka Univers ty, Suita, Osaka, Japan, ++Special Pathogens Branch, Virology Division, Center for Disease Control, Atlanta, USA, +++Special Pathogens Reference Laboratory, PHLS Centre for Applied Microbiology and Research, Porton Down, Salisbury, U.K., ++++The Institute of Poliomyelitis and Viral Encephalitides of the U.S.S.R. Academy of Medical Sciences, Moscow, U.S.S.R.

Received January 16, 1987

Summary. — The antigenic relations among nine Hantaviruses from seven different geographic areas were examined in indirect fluorescent antibody test with 12 monoclonal antibodies. Ten monoclonals were prepared against Hantaan virus strain 76—118 and two against Hantavirus B-1, a virus isolated from a tumour in a laboratory rat. Four different serotypes have been differentiated: Hällnäs, Hantaan, Prospect Hill and Rat strain like. Two monoclonal antibodies were able to react with all nine Hantaviruses studied. Rat strain- and Hantaanvirus-specific monoclonal antibodies were documented.

Key words: Hantaviruses; monoclonal antibody; antigenic relation

Introduction

Haemorrhagic fever with renal syndrome (HFRS) or Hantavirus disease (Desmyter *et al.*, 1984) is a worldwide epidemic disease and is characterized by acute nephritis. Several viruses that are serologically related to Hantaan virus (HTN) have been isolated from various rodents in different regions of Korea (Lee *et al.*, 1982), the People's Republic of China (Song *et al.*, 1982); Song *et al.*, 1984; Yan *et al.*, 1986), Japan (Sugiyama *et al.*, 1984; Kitamura *et al.*, 1983), United States (Le Duc *et al.*, 1983; Tsai, in press), Egypt (Lee *et al.*, in preparation), Brazil (Lee *et al.*, 1984), Hong Kong (Lee *et al.*, 1986). Europe (Lloyd *et al.*, 1986; Niklasson *et al.*, 1984; van der Groen *et al.*, in press; Yanigahara *et al.*, 1984), U.S.S.R. (Tkachenko *et al.*, 1984). For the development of diagnostic tests and vaccines it is necessary to know the common as well as the distinct antigenic determinants of these Hantavirus strains.

[1]) Whom requests for reprints should be addressed to (Dept. Microbiol., Inst. Trop. Med., Nationalestraat 155, B-2000 Antwerp, Belgium)

In the present work the antigenic relations among nine Hantaviruses from different geographic areas were examined by indirect fluorescent antibody test with 12 different monoclonal antibodies.

Materials and Methods

Hantavirus antigen slide preparation. Gammairradiated (3×10^6 RAD) inactivated Vero-E6 cell suspension infected with the different Hantaviruses under study, was prepared as previously described (van der Groen *et al.*, 1985). Indirect fluorescent antibody (FA) assay was performed as previously described (van der Groen *et al.*, 1985). Fluoresceinlabelled sheep anti-mouse IgG (Pasteur) Lot No. 14 was diluted (1 drop/ml) in phosphate buffered saline (PBS) pH 7.2, containing Evans blue at a final concentration of 0.1%. All titre determinations were performed at least twice by two different laboratory workers.

Monoclonal antibodies 1 to 6 (Table 1) were prepared against Hantaan virus and Hantavirus B1 as previously described (Yamanishi *et al.*, 1984). Monoclonal antibodies 7 to 12 (Table 1) were prepared against Hantaan virus by Dr. J. McCormick's group according a Centres for Disease Control standard protocol.

Results and Discussion

All Hantavirus-infected Vero E6 cells used in these experiments were tested on the presence of reoviral antigens using monoclonal antibodies against reoviruses 1, 2 and 3, kindly supplied by Dr. Joklik, Durham University, N. Carolina, U.S.A. No reovirus antigen could be demonstrated. In the past Hantaviruses contaminated with reoviruses had been described (Goldgaber *et al.*, 1982) and therefore, it is always advisable to check for reoviral antigens, when a new Hantavirus agent was isolated. An additional reason to screen for the reovirus antigens in Hantavirus infected E6 cells is the high prevalence of reovirus antibodies in human sera. All monoclonal antibodies used in the comparative study were negative at 1 : 16 dilution when tested by IF assay for reovirus serotypes 1, 2 and 3 in infected Vero E6 cells.

Monoclonal antibodies 1 and 2, prepared by Dr. Yamanishi, showed a broad reactivity in IF assay for all hantaviral antigens tested so far. These monoclonal antibodies could be very suitable for rapid specific detection of hantaviral antigens in cell culture as well as in organs of infected animals. These monoclonal antibodies absorbed on plastic could capture the antigen, the latter being detected by the same or another broad reacting labelled monoclonal antibody. The development of this antigen capturing method is now in progress.

Monoclonal antibodies 1 to 6 allowed to differentiate 4 serotypes in the IF test (Table 1):

1. Nephropathia epidemica (NE) like (CG 13891, CG 18—20,Hälnäss)
2. Prospect Hill (PH) like
3. Hantaan like (HNT, CG 38—83)
4. Rat strain like (SR-11, TCH, GB-B).

Abbreviations used for the different Hantavirus strains are explained in detail in the legend of the Table.

The same serotypes have been demonstrated with neutralization; and radiommunoassay procedures as described elsewhere (Schmaljohn *et al.*,

Table 1. Arithmetic mean of coded titres[a] of monoclonal antibodies made against Hantaviruses on different Hantaviral antigens in the indirect immunofluorescent antibody test

Clone[c])	CG 13891[d])	CG 18—20	NE	PH	HNT	CG 38—83	SR-11	TCH	GB-B
1	3.2 ± 0.1	3.4 ± 0.3	2.8 ± 0.2	3.2 ± 0.4	3 ± 0.1	3 ± 0.3	2.9 ± 0.1	3 ± 0.3	3.7 ± 0.3
2	3 ± 0.2	3.5 ± 0.4	3.4 ± 0.3	3 ± 0.3	3.65 ± 0.05	3.6 ± 0.2	3.4 ± 0.3	3.4 ± 0.3	3.8 ± 0.1
3	2.6 ± 0.4	2.9 ± 0.4	2.6 ± 0.4	—	3 ± 0.3	3 ± 0.3	2.9 ± 0.1	2.8 ± 0.3	2.9 ± 0.1
4	—[b]	—	—	—	—	—	3.8 ± 0.2	3.8 ± 0.2	3.6
5	—	—	—	—	—	—	4.2	3.9 ± 0.3	—
6	—	—	—	—	4.7 ± 0.1	4.85 ± 0.05	—	—	—
7	—	2.2 ± 0.3	—	—	3.6 ± 0.2	3.7	2.4 ± 0.4	2.6 ± 0.7	3.1
8	—	—	—	—	4.7 ± 0.1	4.5 ± 0.2	—	—	—
9	—	—	—	—	3.8 ± 0.1	4	4.55 ± 0.05	3.8 ± 0.1	3.6 ± 0.6
10	—	—	—	—	3.3 ± 0.2	3.4 ± 0.3	—	—	—
11	—	—	—	—	5.3 ± 0.1	5.2	—	2.2 ± 0.3	—
12	—	2.6 ± 0.2	2.2	—	4.85 ± 0.05	5 ± 0.2	4.9 ± 0.4	3.8 ± 0.5	3.3

a) Arithmetic mean of two titrations performed by two different persons. Coded titres are $\log_{10}$ of the inverse of the highest dilution at which still a characteristic fluorescent pattern can be observed.

b) means negative at a dilution of 1/16.

c Monoclonal antibodies 1, 2, 3 and 6 are mouse ascitic fluids obtained from BALB/c mice inoculated with Hantaan virus strain 76—118. The numbers in the table correspond with clone numbers in the following way: 1 = 43 B (G2a); 2 = 133E (G2a); 3 = 141D (G1); 6 = 19B (G1) (subclass of IgG in between brackets). Monoclonal antibodies 4 and 5 are mouse ascitic fluids obtained from BALB/c mice inoculated with virus B-1, isolated from a rat in Japan. These numbers correspond with the clone numbers as 4 = B8 (61); 5 = B61 (G1). Monoclonal antibodies 7 to 12 were prepared against Hantaan strain 76—118 in the laboratory of Dr. J. McCormick, CDC, Atlanta, U.S.A. The numbers in the table correspond with the following hybridoma clone numbers: 7 = FDO3-AA11; 8 = = BDO1-BBO8; 9 = HCO2-BDO5; 10 = KDO1-AD12; 11 = HDO1-ADO2. 12 = ECO2 BDO1.

d) virus strains

CG 13891: virus isolated from lung suspensions of a wild living *Clethrionomys glareolus* captured in Turnhout, Northern part of Belgium. Fourth passage of CG 13891 in Vero E6 cells was used.

CG 18—20 and CG 38—83: viruses isolated from lung suspensions of wild living *Clethrionomys glareolus* captured in the Western part of the U.S.S.R. (Bashkiria), Lot No. 255 21/2/85.

NE: Hällnäs strain isolated from wild living *Clethrionomys glareolus* in Sweden, Lot No. 568/5 21/2/85.

PH: Prospect Hill — PH1 strain isolated from *Microtus pennsylvaticus* in the USA. Lot No. 569/7 21/2/85.

HNT: Hantaan strain 76—118 isolated from *Apodemus agrarius* in Korea. Lot No. 547 21/2/85 and 542 13/5/85.

SR-11: Isolated from a laboratory Wistar rat in Japan. Lot No. SR-11 570/7 21/2/85.

TCH: Tchoupitoulas isolated from the pancreas of a *Rattus norvegicus* captured in the U.S.A. Lot No's 579/5 19/4/85 and 579/5 21/2/85.

GB-B: Isolated from immunocytomas of a laboratory Lou/C rat U. K. Lot No. 601 13/5/85.

1984). Some monoclonal antibodies were more strain specific. For example the monoclonal antibody 4 reacted only with the rat strains. Monoclonal antibody 5 reacted with SR-11 and TCH strains only; monoclonal antibody 10 reacted with Hantaan and CG 38—93 strains only. Monoclonal antibodies 7 to 12 were able to detect minor differences among the main serotypes.

In the NE group it is clear that the Hantavirus isolated in the Western part of the U.S.S.R. (CG 18—20), the Belgian Hantavirus (CG13891) and the Hällnäss strain from Sweden are not identical. These strains were all isolated from the same species *Clethrionomys glareolus*. They all occurred in areas where the mild form of Hantavirus disease has been described before. Among the rat strains minor differences were observed. The Japanese SR-11 strain is very close to the Great Britain GB-B strain. Both were isolated from laboratory rats.

A correlation between the different serotypes of Hantaviruses and the hosts from which they had been isolated was observed, which confirms the data published by Lee *et al.* (1985). However, Hantaan 76—118 and CG 38—83 although isolated from two totally different hosts, could not be differentiated from each other by any of the monoclonal antibodies. Hantaan 76—118 was isolated in an area where the severe form of Hantavirus disease was described (Korean haemorrhagic fever) and CG 38—83 was isolated in the Bashkiria area (U.S.S.R.) where predominantly the mild form of Hantavirus disease did occur. Further studies are necessary.

Acknowledgements. The authors are indebted to Mrs. G. Beelaert and Mr. G. Hoofd for technical assistance. Mrs. Y. Baeten is greatly acknowleged for typing the manuscript. This work was supported by Fonds Geneeskundig Wetenschappelijk Onderzoek no. 3.0082.86.

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