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The antigenic analysis of haemorrhagic fever with renal syndrome viruses in China by monoclonal antibodies

By Z. K. XU, X. L. AN and M. X. WANG

Department of Microbiology and Laboratory 403-3, The Fourth Military Medical College, Xian, Shaanxi, China

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SUMMARY

Thirty-six strains of haemorrhagic fever with renal syndrome (HFRS) virus were isolated from patients and a number of host animals in various areas in China. They were analysed by an immunofluorescence test (IFAT) using 10 monoclonal antibodies (McAbs) specific for the HFRS virus; antigenic differences among the strains have been demonstrated. The HFRS virus strains revealed nine different reactions with the McAbs, showing that there are at least nine different antigenic determinants including group-, type- and strain-specific. Analysis of the results shows that antigenic differences among the HFRS virus strains are mainly related to differences in the host animals.

INTRODUCTION

Many strains of haemorrhagic fever with renal syndrome (HFRS) virus have been isolated from *Apodemus agrarius*, *Rattus norvegicus*, *A. speciosus*, experimental rat, cat and HFRS patients in various areas in China (Fig. 1) (Song *et al.* 1982*a*, *b*; Ni *et al.* 1983; Li *et al.* 1983*a*; Zhu *et al.* 1983; Luo *et al.* 1985). These virus strains have physical, chemical and morphological characteristics similar to each other (Hung *et al.* 1983) and cannot be differentiated by indirect immunofluorescence antibody technique (IFAT) with serum from convalescent HFRS patients or animal antisera. Different clinical symptoms and epidemiological characteristics are seen in cases of HFRS presenting in different areas of China (Yan *et al.* 1982; Xu *et al.* 1982; Li *et al.* 1983*b*). These findings suggest that there are antigenic differences among the HFRS virus strains. Clarification of this will be helpful in aetiological and epidemiological studies of HFRS and in the preparation of an HFRS vaccine.

This paper reports the results using IFAT with monoclonal antibodies (McAbs) directed against HFRS virus in an antigenic analysis of HFRS virus strains isolated from various areas in China.

Address for correspondence: Dr Hong yi Zhang, Experimental Pathology, Floor A, St Mary's Hospital Medical School, Praed Street, London W2 INY.

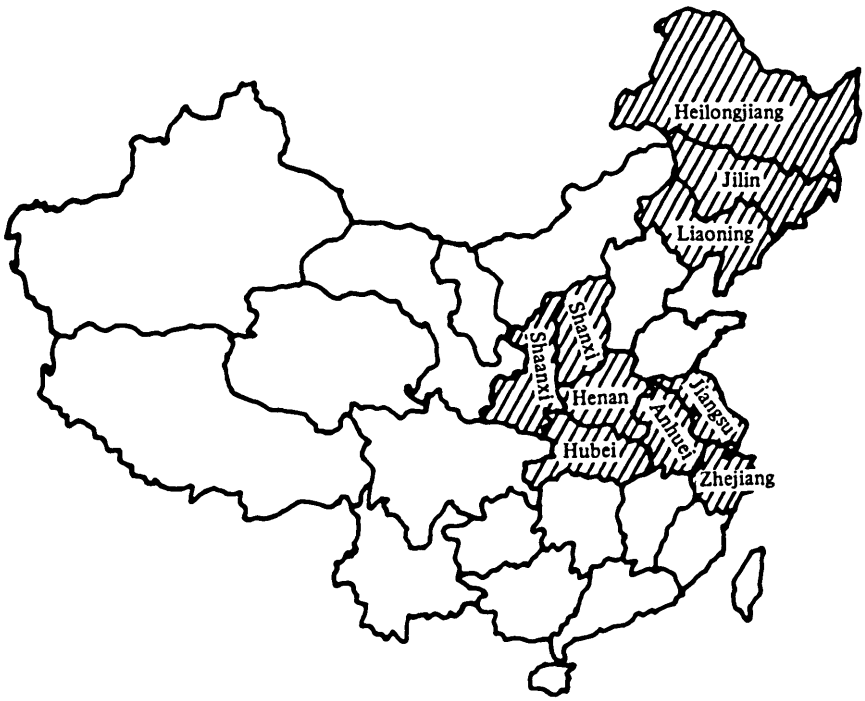

Fig. 1. The map of China: ▨ provinces where the HFRS virus strains in this study were isolated.

MATERIALS AND METHODS

Monoclonal antibodies specific for HFRS virus

The 10 McAbs used in the study were produced by hybridoma cell lines which were established by fusion of Sp2/0 mouse myeloma cells with spleen cells of BALB/C mice immunized with HFRS virus strain 82–010H, (An *et al.* 1984).

HFRS virus strains

Thirty-six strains of HFRS virus were isolated from patients and several host animals in various areas in China and one strain (76-118) was isolated from South Korea (Lee, Lee & Johnson, 1978). Vero-E6 cells, A 549 cells or suckling mouse brains infected by the above 37 strains of HFRS virus were prepared for antigenic analysis. The passage history of these strains is shown in Table 1.

Immunofluorescent antibody test (IFAT)

HFRS virus-infected cells or brain tissue sections were fixed to microscope slides (Table 1) with acetone for 10 min at 4 °C, washed with 0.01 M phosphate-buffered saline (PBS, pH 8·0), and then reacted with the McAb (1/50) or a control rabbit-anti-HFRS virus serum for 45 min at 37 °C. After being washed three times, the cells or the tissue sections were reacted with fluorescein-labelled rabbit-anti-BALB/C mouse IgG or goat anti-rabbit globulin (Beijing Institute of Biological Products, China) for 30 min at 37 °C, rewashed three times and examined with a Leitz microscope fitted with epi-fluorescence.

Table 1. *Passage histories of* 36 *HFRS viruses used in this study*

Virus isolates	Cells for virus isolation	Viral antigen slides	No. of passages
82–010H	Vero-E6	Vero-E6 cell slides	7
Fli	..	..	6–7
Fk	..	..	6–7
Flu	..	..	6–7
14A	*Ap. a.* (lungs)	..	10
A96	A 549	A 549 cell slides	8
A41	..	..	NC
S2	..	..	8
Chen	..	..	7
Hu	..	..	7
Li	..	..	7
C4	Vero-E6	Vero-E6 cell slides	7
A1	*Ap. a.* (lungs)	..	10
A5	..	..	10
A9	..	..	10
R4	Vero-E6	..	5
R1	..	..	5
R2	..	..	5
R3	..	..	5
R5	..	..	5
R6	..	..	5
ZR3	*Ap. a.* (lungs)	*Me. u.* brain sections	NC
S83011	Vero-E6	Vero-E6 cell slides	8
A54	..	..	8
H8235	..	..	10
H8278	..	..	10
H8205	..	..	10
R22	..	..	9
R27	..	..	9
Hs5	NC	..	NC
Rn4	Vero-E6	..	7–9
Rn15	..	..	7–9
Hb3	..	..	7–9
H214	..	..	7–9
Hu9	..	..	NC
Hu11	..	..	NC

Ap. a, *Apodemus agrarius*; *Me. u*, *Merionea unguiculatus*; NC, not clear.

All the viruses isolated in cell cultures were not passaged in animals. All the viruses isolated in *A. agrarius*, except for ZR3, were not passaged in other species of animals, but adapted to cell cultures.

RESULTS

As shown in Table 2, 9 different immunofluorescence patterns were produced with the 10 McAbs tested on 36 strains of HFRS virus. The results demonstrate that there are at least nine different antigenic determinants on HFRS virus.

McAb4E7 reacted with all the strains of HFRS virus tested. This shows that it is directed against the group-specific antigenic determinant 1. McAb5H5 reacted predominantly with strains isolated from *Apodemus agrarius*, *A. speciosus* and patients from the areas where the host animals and sources of infection were mainly both types of apodemus. This therefore suggests that McAb5H5 is directed against the apodemus type-specific antigenic determinant 4. McAb4B9 and 4E8

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Table 2. *Immunofluorescent reactions of McAbs upon HFRS virus strains*

Areas	Virus strains and sources	McAbs 24E7	4B9	4E8	5H5	3D10	3G4	3H4	4H7	3B3	3F9	Control anti-serum (rabbit)
South Korea	76–118(*Ap.a.*)	:::	:::	:::	:::	:::						:::
Shannxi	82–010H(Pat.)	:::	:::	:::	:::	:::	:::	:::	:::	:::	:::	:::
	Fli(Pat.)	:::	:::	:::	:::							:::
	Fk(Pat.)	:::	:::	:::	:::							:::
	Flu(Pat.)	:::	:::	:::	:::							:::
	14A(*Ap. a*)	:::	:::	:::	:::							:::
Anhuei	A96(*Ap. a.*)	:::	:::	:::	:::							:::
	A41(*Ap. a.*)	:::	:::	:::	:::							:::
	S2(Pat.)	:::	:::	:::	:::							:::
	Chen(Pat.)	:::	:::	:::	:::							:::
	Hu(Pat.)	:::	:::	:::	:::							:::
	Li(Pat.)	:::	:::	:::	:::							:::
	C4(Cat)	:::	:::	:::								:::
	A1(*Ap. a.*)	:::	:::	:::	:::							:::
	A5(*Ap. a.*)	:::	:::	:::	:::							:::
	A9(*Ap. a.*)	:::	:::	:::	:::							:::
	R4(Rat)	:::	:::	:::	:::							:::
Jiangsu	R1(Rat)	:::	:::	:::								:::
	R2(Rat)	:::	:::	:::								:::
	R3(Rat)	:::	:::	:::								:::
	R5(Rat)	:::	:::	:::								:::
	R6(Rat)	:::	:::	:::								:::
Zhejiang	ZR3(*Ap. a.*)	:::	:::	:::	:::	:::						:::
Liaoning	S83011(Pat.)	:::	:::		ND		:::					:::
Jilin	A54(*Ap. s.*)	:::	:::	:::	:::							:::
	H8235(Pat.)	:::			:::							:::
	H8278(Pat.)	:::			:::							:::
Heilong-Jiang	H8205(Pat.)	:::			:::							:::
		:::			:::							:::
Henan	R22(*Ra. n.*)	:::										:::
	R27(*Ra. n.*)	:::	:::									:::
	Hs5(Pat.)	:::										:::
Shanxi	Rn4(*Ra. n.*)	:::				:::	:::	:::	:::			:::
	Rn15(*Ra. n.*)	:::				:::	:::	:::	:::			:::
	Hb3(Pat.)	:::				:::	:::	:::	:::			:::
	H214(Pat.)	:::				:::	:::	:::	:::			:::
Hubei	Hu9(pat.)	:::				:::	:::	:::	:::	:::		:::
	Hu11(Pat.)	:::				:::	:::	:::	:::	:::		:::
Antigenic determinants		1	2	3	4	5	6	7		8	9	

:::, positive reaction; empty area negative reaction; *Ap.a*, *Apodemus agrarius*; Pat., patient; *Ap. s. Apodemus speciosus*; *Ra.n*, *Rattus norvegicus*; ND, not done.

reacted mainly with the strains isolated from Shaanxi, Anhuei, Jiangsu and Zhejiang Provinces, where *A. agrarius* was the main host animal and therefore source of infection. Here the suggestion is that both of the McAbs are directed against *A. agrarius* type-specific antigenic determinants 2 and 3. Except for the strain R27 from Henan, the strains of HFRS virus isolated from Henan, Shanxi

and Hubei Provinces, where *Rattus norvegicus* is the main host animal and source of infection, did not react with McAbs4B9, 4E8 or 5H5. The remaining Henan strains, R22 and Hs5, reacted only with McAb4E7, and the strains from Shanxi and Hubei reacted not only with McAb4E7 but also with McAb 3D10, 3G4, 3H4 and 3H7, and 3B3 (i.e. antigenic determinants nos 5, 6, 7 and 8 respectively). McAb3F9 reacted only with strain 82–010H which was used for the preparation of this McAb, and suggests that it is probably directed against the strain-specific antigenic determinant 9.

DISCUSSION

It is known that the spread of HFRS in various areas in China is caused by HFRS virus, but the sources of infection, clinical symptoms and epidemiological characteristics of the HFRS vary in these areas (Yan *et al.* 1982; Xu *et al.* 1982; Li *et al.* 1983*b*) suggesting that there are antigenic differences between the strains of virus. The antigenic difference between those from wild species of mouse and most house rats was shown by using cross-IFAT, cross-neutralization and cross-blocking tests (Song *et al.* 1984). This has also been clearly shown by Schmaljohn *et al.* (1985) and Goldgaber *et al.* (1985). They defined three antigenic groups or serotypes by analysing the cross-reactivity of virus isolates in radio- and immuno-assays. More recently, Lee *et al.* (1985) defined four serotypes of the viruses by using cross-indirect immunofluorescent antibody and plaque reduction neutralization tests. Serotype 1 included strains derived from *Apodemus* spp., serotype 2 included strains derived from *Rattus* spp., serotype 3 included strains derived from *Clethrionomys* spp. and serotype 4 included strains from *Microtus* spp. Franko *et al.* (1983) prepared six McAbs specific for HFRS virus strain 76-118, three of which were capable of distinguishing the 76-118 and Lee strains of HFRS virus. Recently, Chen *et al.* (1985) analysed the antigenicity of the HFRS virus strains by McAbs.

The present study, using an IFAT with 10 McAbs directed against HFRS virus, analyses the antigenic specificity of 36 strains of HFRS viruses isolated from various areas in China and one strain from South Korea. The results demonstrate nine different reactive patterns with the McAbs, showing that there are at least nine different antigenic determinants in the HFRS virus (see Table 2).

Antigenic determinant 1, which is shared by all the 36 strains, is probably group-specific for HFRS virus. It is probably represented in the serotype 1 and 2 viruses of Lee *et al.* (1985). We do not know how widely this antigen is distributed because *Clethrionomys*- and *Microtus*-derived viruses, serotypes 3 and 4, were not included in this study. The strains isolated from *Apodemus agrarius* and from patients in the areas where the host animal and source of infection is mainly *A. agrarius*, and where the symptoms of the patients are more serious, have less distinct antigenic differences and they possess mainly antigenic determinants 1–4, which may be shared by the serotype 1 viruses of Lee *et al.* This shows that the antigenic specificity of the strains carried by *A. agrarius* is comparatively stable. The strains isolated from a cat and experimental rats in the areas described above have antigenic determinants 1–3, but not 4. It can be assumed that the strains carried by both these species of animal were probably from *A. agrarius* and that, in the course of spreading, certain changes have occurred to antigenic determinant

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4. It was noticed that the antigenic specificity of strain ZR3 (Zhu *et al.* 1983). isolated from *A. agrarius* in Zhejian, was similar to that of strain 76–118 from *A. agrarius* from South Korea.

The antigenic specificity of the strains isolated from the areas where the host animal and source of infection is mainly *Rattus norvegicus* and the symptoms of the patients are comparatively less severe is different from that of the strains described above. Except for one strain, none has antigenic determinants 2–4. With the one exception, all strains from Henan have only antigenic determinant 1. The strains from Shanxi have 1 and 5–7, and the strains from Hubei have 1 and 5–8. These results show that the antigenic differences of the strains carried by *R. norvegicus* are comparatively distinct. There may be antigenic determinants 5–8 in some of the serotype 2 viruses of Lee *et al.*

It was shown that there was a greater antigenic difference between the strains isolated from areas where *Apodemus agrarius* was the natural host and those isolated from *A. speciosus* areas, although both of the rodents were variants of *Apodemus*. The strains from the *A. speciosus* area possess antigenic determinants 1 and 4, but do not have 2 and 3. These results show that it is not possible to distinguish types of HFRS virus according to the host animal from which the virus strain is isolated.

It is worth noting that the strains isolated from the patients with HFRS in a certain area possess a similar antigenic specificity to the strains from the principal host animal in the same area (see Table 1). This finding suggests that these antigenic differences could account for the variations in severity of the HFRS and the different clinical symptoms and epidemiological characteristics. Of course, this has to be further verified by studying the virulence and other characteristic HFRS virus strains possessing different antigenic specificity.

It is necessary to study the spread of the virus in different host animals, the strain variation and the antigenic determinant in relation to the neutralizing antibody.

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