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BRIEF COMMUNICANION

ENZYME-LINKED IMMUNOSORBENT ASSAY FOR DETECTION OF ANTIBODY AGAINST HEMORRHAGIC FEVER WITH RENAL SYNDROME VIRUS IN SERA FROM EXPERIMENTALLY INFECTED RATS.[1]

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Hemorrhagic fever with renal syndrome (HFRS) is a serious zoonotic disease transmitted by various kinds of rodents in many areas of the world. In Japan, several HFRS virus strains were isolated from laboratory rats[6] and also from urban rats captured in reclaimed areas.[1,2] Human cases of HFRS in Japan have been acquired mostly from laboratory rats infected with the virus. Thus, the infected rats have to be detected and eliminated by reliable diagnostic tests. Methods of isolation of the virus or detection of viral antigen in the rats are time and labor consuming and have limited sensitivity. The detection of antibody is more practical and useful for diagnostic purposes. Serological tests for HFRS virus antibody include the immunofluorescent antibody (IFA) test, immune adherence hemagglutination (IAHA) test, complement fixation (CF) test and neutralization (NT) test. Enzyme-linked immunosorbent assay (ELISA) has been applied in detecting antibodies against many kinds of viruses. In this report, ELISA was evaluated for its ability to detect HFRS virus antibody in experimentally infected rats by comparing it with the serological tests listed above.

HFRS virus SR–11 strain isolated from laboratory rats[6] was propagated in a Vero E–6 cell monolayer and used in the study. Four 5–week–old SPF Wistar rats were inoculated subcutaneously with SR–11 Strain (1×10^6 FFU). Blood was collected at intervals by cutting the tails of the rats and used for obtaining serum or applied on filter paper. Virus antigen for ELISA, IAHA and CF tests was prepared by inactivating the virus in Vero E–6 cells with β-propiolactone (0.2%) and performing ultrasonic

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treatment (100W for 10min) of inactivated infected cells. ELISA procedure was essentially the same as previously described,[7,8] and the minor changes were as follows: 1) anti-rat IgG and -rat IgM conjugated with horseradish peroxidase were used as the conjugates, and 2) azinodi-3-ethylbenzthiazoline sulfonic acid was used as the substrate.[3] Specificities of the conjugates were confirmed by reacting the conjugates against IgG or IgM fraction of the sera fractionated by ultracentrifugation. IFA and IAHA procedures were followed as described before.[2] NT antibodies were assayed using 50% focus reduction method which was a modified aasay of fluorescent focus counting described before.[9] CF titers were assayed by 50% hemolysis method.[4]

HFRS antibody titers were examined at intervals in the sera from rats experimentally infected with the virus. Titers of ELISA IgG, ELISA IgM and NT antibodies are shown in Fig. 1. ELISA IgG or IgM titers were equal to or less than 1:12.5 in the sera before virus inoculation in 4 rats, and NT titers were less than 1:10. ELISA IgM antibodies were first detected in 4 rats on day 5 or 7 after virus infection, reached a maximum titer between day 5 and 10, decreased rapidly down to 1:50 or less on day 12 and were constantly low up to day 42. ELISA IgG antibodies were first detected in 3 of 4 rats on day 5, increased in titers rapidly, reached maximum titers on day 21 and were maintained at high titers up to day 42. NT titers

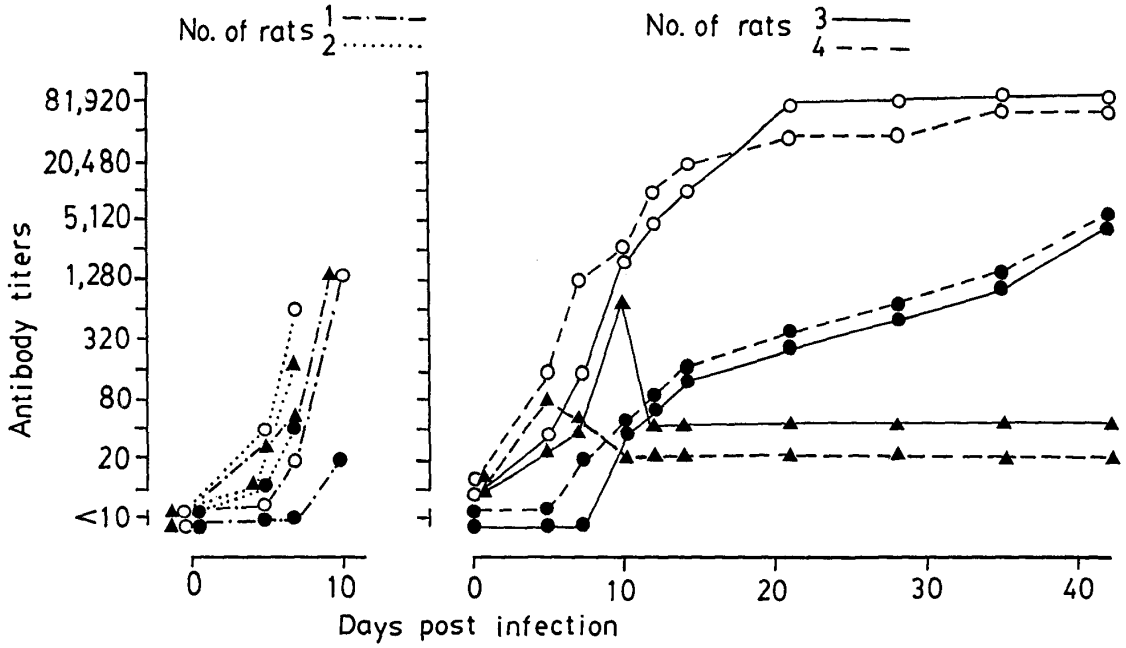

FIGURE 1 Titers of ELISA IgG, ELISA IgM and NT antibodies to HFRS virus in experimentally infected rats. Symbols represent the antibody titers in ELISA IgG (○), ELISA IgM (▲) and NT (●) in 4 rats

TABLE 1 Titers of ELISA, IFA, CF and NT antibodies to HFRS virus in experimentally infected rats

Test	Rat No.	Antibody titers[a] on day					
		0	5	7	10	21	42
ELISA	1	< 12.5	< 12.5	25	1,600(3,200)[b]	ND[c]	ND
	2	12.5	12.5	50	800	ND	ND
	3	12.5	50	200	3,200(6,400)	102,400	102,400(102,400)
	4	12.5	200	1,600	3,200(6,400)	51,200	102,400(102,400)
IFA	1	< 16	< 16	< 16	512(512)	ND	ND
	2	< 16	32	256	ND	ND	ND
	3	< 16	< 16	32	1,024(1,024)	4,096	8,192(8,192)
	4	< 16	< 16	125	512(512)	2,048	4,096(4,096)
IAHA	1	< 4	< 4	< 4	256(512)	ND	ND
	2	< 4	16	64	ND	ND	ND
	3	< 4	< 4	< 4	512(1,024)	8,192	16,384(16,384)
	4	< 4	< 4	256	512(512)	4,092	8,192(8,192)
CF	1	< 4	< 4	< 4	32(64)	ND	ND
	2	< 4	8	16	ND	ND	ND
	3	< 4	< 4	< 4	256(256)	2,048	4,096(4,096)
	4	< 4	< 4	64	256(256)	1,024	2,048(2,048)
NT	1	< 10	< 10	< 10	20	ND	ND
	2	< 10	10	40	ND	ND	ND
	3	< 10	< 10	< 10	40	320	5,120
	4	< 10	< 10	20	40	320	5,120

[a] Expressed as a reciprocal of serum dilution.
[b] Numbers in a parentheses are the titers in the blood obtained by filter paper.
[c] Not determined.

were detected in 1 rat on day 5 and in 2 rats on day 7, and the titers were gradually and continuously increased up to day 42. Gradual rise of NT titers was also reported in the sera from the mice experimentally infected with HFRS virus.[5)]

Antibody titers were also determined by IFA, CF, NT tests and compared with those in ELISA (IgG) on several days post infection (Table 1). On day 5 after virus infection, antibodies were detected in 3 of 4 rats by ELISA and in 1 of 4 rats by IFA, IAHA, CF and NT tests. On day 7, antibodies were detected in all 4 rats by ELISA, in 3 of 4 rats by IFA test and in 2 of 4 rats by IAHA, CF and NT tests. On day 7, all rats had detectable antibodies in all five tests. Antibody titers were detectable up to 42 days in five tests. Antibodies in the reconstituted blood from filter paper were also detected with almost the same patterns as those of ordinary serum. Day of appearance and presence of the antibody titers were almost the same both in ordinary serum and in reconstituted filter blood. Only the titers on day 10 and day 42 are shown in Table 1.

These results indicated that ELISA was applicable in testing HFRS antibodies in sera from infected rats as well as the other four tests and that it could detect the antibodies at an early stage of infection. Antibody was also detectable by ELISA in reconstituted blood on filter paper. It was concluded that this assay system is a useful tool to monitor the HFRS virus among laboratory rats using only a small amount of blood.

REFERENCES

1) ARIKAWA, J., TAKASHIMA, I., HASHIMOTO, N., MORITA, C., SUGIYAMA, K., MATSUURA, Y., SHIGA, S. & KITAMURA, T. (1985): Epidemiological study of hemmorhagic fever with renal syndrome related virus infection among urban rats in two islands in Tokyo bay in Japan. *Acta Virol.*, **29**, 66–72

2) ARIKAWA, J., TAKASHIMA, I., HASHIMOTO, N., TAKAHASHI, K., YAGI, K. & HATTORI, K. (1986): Epidemiological studies of hemorrhagic fever with renal syndrome (HFRS) related virus infection among urban rats in Hokkaido, Japan. *Arch. Virol.*, **88**, 231–240

3) CHANG, H-C., TAKASHIMA, I., ARIKAWA, J. & HASHIMOTO, N. (1984): Biotin-labeled antigen sandwich enzyme-linked immunosorbent assay (BLA-S-ELISA) for the detection of Japanese encephalitis antibody in human and a variety of animal sera. *J. Immunol. Methods*, **72**, 401–409

4) INOUE, S. (1973): A complement fixation test with the micro titer method. *J. Med. Technol.*, **17**, 838–847 (in Japanese)

5) KIM, G. R. & MCKEE, K. J. JR. (1985): Pathogenesis of Hantann virus infection in suckling mice: clinical and virologic, and serologic observations. *Am. J. Trop. Med. Hyg.* **34**, 388–395

6) KITAMURA, T., MORITA, C., KOMATSU, T., SUGIYAMA, K., ARIKAWA, J., SHIGA, S., TAKEDA, H., AKAO, Y., IMAIZUMI, K., OYA, A., HASHIMOTO, N. & URASAWA, S. (1983):

Isolation of virus causing hemorrhagic fever with renal syndrome (HFRS) through a cell culture system. *Jpn. J. Med. Sci. Biol.*, **36**, 17–25

7) MIYATA, K., UEDA, M. & HASHIMOTO, N. (1982): Antibody assay for Japanese encephalitis virus in bovine serum by enzyme-linked immunosorbent assay. *Jpn. J. Vet. Res.*, **30**, 59–67

8) OHKUBO, Y., TAKASHIMA, I., HASHIMOTO, N. & FUJITA, I. (1984): Enzyme-linked immunosorbent assay (ELISA) for detection of IgM and IgG antibodies to Japanese encephalities virus in sera from experimentally infected swine. *Jpn. J. Vet. Sci.*, **46**, 57–64

9) WHITE, J. P., SHIREY, F. G., FFRENCH G. R., HUGGINS, J. W., BRAND, O. M. & LEE, H. W. (1982): Hantann virus, aetiological agent of Korean hemorrhagic fever, has Bunyaviridae-like morphology. *Lancet* i, 768–771