Antigenic Differences Between Two Viruses, Isolated in Japan and Korea, That Cause Hemorrhagic Fever with Renal Syndrome

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Hantaan virus (HV) 76-118, isolated from Apodemus agrarius coreae in Korea, and hemorrhagic fever with renal syndrome (HFRS) virus B-1, isolated from a rat in Japan, were examined for polypeptide compositions and for differences in immune responses in rats. In immunoprecipitation experiments, a major polypeptide of ca. 50 kilodaltons (K) was detected with antisera against HV 76-118 in cell extracts from Vero E6 cells infected with HFRS virus B-1, whereas three major polypeptides of 74 K (glycosylated), 57 K (glycosylated), and 50 K were detected with antisera against HFRS virus B-1. On the other hand, two polypeptides with molecular weights of 55,000 (glycosylated) and 50,000 were detected with either antiserum in cell extracts infected with HV 76-118. In neutralizing antibody tests with antisera prepared in rats, a remarkable difference in antibody titre (5 to 30 times higher for the homologous virus than to the heterologous virus) was observed between the two viruses. However, this difference was not so marked (1 to 4 times higher to the homologous virus) in the immunofluorescent antibody test. Twenty hybrid cell lines producing mouse monoclonal antibodies against HV 76-118 were isolated by fusion of spleen cells from BALB/c mice immunized against HV (strain 76-118) with mouse myeloma cells. The specificity of these monoclonal antibodies was established by immunofluorescent antibody, neutralizing antibody, and fluorescent antibody to membrane antigen tests and by analysis with sodium dodecyl sulfate-polyacrylamide gel electrophoresis. These hybrid cell lines were classified into three groups based principally on the IF staining pattern of the HV-infected cells: (i) antibodies which showed a discrete patch pattern in the cytoplasm by the immunofluorescent antibody test, reacting with the membrane antigen of infected cells and immunoprecipitating a 55-K glycoprotein from HV 76-118-infected cell lysates and a 57-K glycoprotein from the heterologous (strain B-1) HFRS virus-infected cell lysates. Among these, depending on the neutralizing antibody activity and the reaction with the heterologous antigen, three subgroups designated I-A, I-B, and I-C were established; (ii) antibodies which showed large granular dots in the cytoplasm, neither having neutralizing antibody activity nor immunoprecipitating any antigen; and (iii) antibodies which showed defined granular dots throughout the cytoplasm, reacting with a 50-K polypeptide of both virus strains. These antibodies also classified into two subgroups based on the reactivity with the B-1 strain. These results suggest that there are definite differences in the antigenic properties of HV (strain 76-118) and HFRS virus (strain B-1).

Hemorrhagic fever with renal syndrome (HFRS) is clinically characterized by acute tubular and interstitial nephritis and has a broad geographic distribution (10). In Japan, the first cases of this disease were reported in 1964 (23), and more than 100 researchers using experimental rats have contracted HFRS in the last ten years, and unfortunately, one case was fatal (8). The etiological agent of Korean hemorrhagic fever, named Hantaan virus (HV), was isolated by Lee et al. (11) from Apodemus agrarius coreae, and later it was adapted in tissue culture cells (5). Thereafter, HFRS viruses, which were antigenically related to HV, were isolated in the United States (13) and China (21) from wild or laboratory rats. In Japan, it was first reported that HFRS virus SR-11 was isolated from the homogenate of a lung of a laboratory rat (9), and subsequently, we have been able to independently isolate an HFRS virus (strain B-1) from a tumor specimen of a Fischer rat by explantation into tissue culture (26). Although these viruses were closely related antigenically by the immunofluorescent antibody (IF) test, the neutralizing antibody (NT) test made the antigenic differences clear (14). It is of interest to compare these viruses more precisely with regard to their antigenic properties.

In this study, we report the difference in polypeptides of HV (strain 76-118) and HFRS virus (strain B-1) on virological and immunological properties. Furthermore, we have isolated mouse hybridomas which secrete monoclonal antibodies reacting with the HV antigen, and using monoclonal antibodies, we also have compared two HFRS viruses isolated in Korea and Japan with respect to their antigenic properties.

MATERIALS AND METHODS

Cells and viruses. Vero E6 cells were obtained from the American Type Culture Collection and grown in medium containing a mixture of medium 199 and Eagle minimal essential medium supplemented with 10% fetal calf serum (FCS) for growth medium and 3% FCS for maintenance medium. SP2/0-Ag14 mouse cells were cultured in Dulbecco modified Eagle medium (DMEM) supplemented with 15% FCS and 20 μg of 8-azaguanine per ml.

HV 76-118 was obtained from the American Type Culture Collection and passaged in Vero E6 cells. HFRS virus B-1, which was isolated in our laboratory from a tumor specimen of a rat, was also passaged in Vero E6 cells (26). Stock viruses were prepared as follows. Vero E6 cells were
infected with HV 76-118 at a multiplicity of infection of 0.01 50% tissue culture infective dose (TCID₅₀) per cell and cultured for 2 weeks. Cultures were subjected to freeze-thawing three times and centrifuged at 3,000 rpm at 4°C for 10 min. Supernatants were stored at -80°C. Virus titer was assayed by indirect IF test as described by Lee et al. (11). Briefly, Vero E6 cells were seeded in eight-chamber slides (Miles Scientific, Div. of Miles Laboratories, Inc.), and virus was inoculated onto slides. Cultures were kept at 37°C for 2 weeks, fixed in cold acetone, and stained with sera from convalescent patients.

Antisera. Antisera against HV 76-118 and HFRS virus B-1 were prepared in Fischer rats. Rats were subcutaneously inoculated with 0.5 ml of stock virus of HV 76-118 (1.5 × 10⁴ TCID₅₀ per ml) and HFRS virus B-1 (7.5 × 10⁴ TCID₅₀ per ml) and bled 6 weeks after injection. Human convalescence phase sera were obtained from patients in Osaka, Japan.

Immunization of mice. A suspension of HV 76-118 was inoculated into cultures of Vero E6 cells for preparation of the HV antigen. In 2 weeks, they were harvested by scraping, washed several times in phosphate-buffered saline (PBS) by centrifugation at 4°C, and suspended in 10 to 20 ml of PBS. Cells were disrupted in a sonicator, and cell extracts were stored at -80°C until use. Inbred BALB/c mice were immunized intraperitoneally with 0.5 ml of HV-infected cell extract in Freund complete adjuvant per mouse. One month later, the mice were boosted with the same antigen without adjuvant by intraperitoneal inoculation, and spleens were removed 3 days after the booster for the preparation of hybridomas.

Isolation of hybrid cells. Procedures for producing hybrid cell lines were essentially the same as those described by Oi et al. (19) and Goding (7). Briefly, spleen cells were washed with DMEM by centrifugation at 1,000 rpm for 5 min at room temperature and suspended in a hemolyzing solution (155 mM NaCl, 10 mM KHCO₃, 1 mM sodium EDTA) for 10 min in ice to lyse the erythrocytes. Cells were centrifuged at 1,000 rpm for another 5 min at 4°C and washed with DMEM twice. The spleen cells and SP2/0-Ag14 mouse cells were mixed at a ratio of 10 to 1 and centrifuged again at 1,000 rpm. The cells were fused by adding 50% polyethylene glycol 4,000 (Nakarai Chemicals, Kyoto, Japan), washed once with DMEM supplemented with 10% FCS, and pelleted by gentle centrifugation once. They were suspended in DMEM with 15% FCS, and 100 μl of the cells suspension was added directly to each well containing HAT (hypoxanthine-aminopterin-thymidine) medium (7). Then one-half of the medium was removed and replaced with fresh HAT medium every other day. About 10 days later, the medium was changed to HT (hypoxanthine-thymidine) medium (7), and 1 week later, the medium was changed to DMEM supplemented with 15% FCS. The culture fluids from viable hybridomas were screened for the secretion of immunoglobulins against HV by the indirect IF technique, and hybridomas scored as positive were transferred to 24-well plastic trays. The hybridomas producing antibody were cloned twice by the limiting-dilution method on feeder layers of mouse normal spleen cells in 96-well trays. The clones which continued to produce antibodies were expanded and prepared for injection into mice for propagation as ascites.

Production of ascites. Mice were primed by intraperitoneal injection with 0.5 ml of pristane (2,6,10,14-tetramethylpentadecane, Aldrich Chemical Co.) 10 days before injection of cloned hybrid cells. About 10⁶ hybridoma cells were inoculated into mice intraperitoneally, and ca. 3 to 5 ml of ascitic fluid was usually trapped from the abdomen at 10 to 14 days after inoculation.

Labeling of viral polypeptides in infected cells and SDS-PAGE after immunoprecipitation. Monolayers of Vero E6 cells in plastic plates (100 mm) were infected at a multiplicity of ca. 0.05 TCID₅₀ per cell. The virus was allowed to adsorb to the cells for 1 h at 37°C, and cultures were kept in an atmosphere of 5% CO₂ at 37°C. At 7 to 9 days postinfection, the medium was removed, and the cells were washed with PBS three times. The cells were cultured in methionine-free MEM supplemented with 2% FCS serum and [³⁵S]methionine (10 μCi/ml; specific activity, 1,445 Ci/mmole; Amersham Corp.) or Eagle minimal essential medium supplemented

FIG. 1. Fluorogram of SDS-PAGE. Cell lysates from HFRS virus B-1 (lanes a to c and g to i) and HV 76-118 (lanes d to f and j to l) cultures labeled with [³⁵S]methionine (lanes a to f) or [³H]mannose (lanes g to l) were reacted with antisera against HFRS virus B-1 (lanes a, d, g, and i), HV 76-118 (lanes b, c, h, and k), or normal sera (lanes c, f, i and l); immunoprecipitates were analyzed by SDS-PAGE.
with [3H]mannose (50 μCi/ml; specific activity, 44.5 Ci/mmol, Amersham) for 8 h. Cells were washed with cold PBS, scraped from the surface of plastic plates, and suspended in RIPA buffer (pH 7.4) 0.01 M Tris-hydrochloride 0.15 M NaCl, 1% sodium deoxycholate, 1% Triton X-100, 0.1% sodium dodecyl sulfate (SDS), 1 mM methionine, 1 mM phenylmethylsulfonyl fluoride). Then cells were disrupted in a sonicator, kept in ice water for 1 h, and centrifuged at 38,000 rpm for 2 h at 4°C.

The antiserum or supernatants of hybridomas were used for immunoprecipitation. A 400-μl portion of each extract, solubilized in RIPA buffer, was incubated with 10 μl of antiserum or 2 μl of monoclonal antibodies for 18 h at 4°C. The immunoglobulins were then absorbed with protein A-Sepharose CL-4B beads (Pharmacia Fine Chemicals) and washed with RIPA buffer. The washed pellets were resuspended in 200 μl of sample buffer (pH 8.2), 0.125 M Tris, 1% SDS, 3% β-mercaptoethanol, 15% glycerol) and boiled for 3 min.

After being centrifuged at 5,000 rpm for 2 min, the supernatants were analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) by a method described elsewhere (17). The reference 14C-methylated protein mixture contained (molecular weight): myosin (200,000), phosphorylase b (92,500), bovine serum albumin (66,000), ovalbumin (45,000), carbonic anhydrase (30,000), and lysozyme (14,300). This mixture was purchased from Amersham.

**FAMA test.** Membrane staining for the fluorescent antibody to membrane antigen (FAMA) test was substantially the same as that described by Franko et al. (4). Briefly, Vero E6 cells infected with strain B-1 were trypsinized and suspended in growth medium. Living cells (ca. 10⁶ cells) were mixed with the monoclonal antibodies (1:100 diluted ascitic fluid), kept at 37°C for 1 h, and washed five times with PBS by centrifugation (2,000 rpm, 5 min). Then, cells were mixed again with anti-mouse immunoglobulin G rabbit serum conjugated with fluorescein isothiocyanate (Cappel Laboratories), and the mixtures were reacted at 37°C for an additional hour. Then, they were washed with PBS, and the cells were observed under the fluorescent microscope.

**NT test.** For the NT test, serial dilutions of antisera or monoclonal antibodies (0.1 ml) were mixed with 0.1 ml of stock virus (about 100 TCID₅₀ per 0.1 ml) and kept at 37°C for 1 h. One-tenth aliquots of this mixture were inoculated on Vero E6 cells grown in eight-chambered slides. At 5 days postinfection, cultures were fixed with cold acetone and stained by indirect IF test. Antibody titers represent the reciprocal value of the dilution of antibody causing a 50% reduction in focus formation.

**RESULTS**

**Identification of HFRS virus polypeptides.** When cell extracts infected with strain B-1 of HFRS virus and labeled with [3H]methionine were immunoprecipitated with rat immune sera against the homologous virus strain, polypeptides of ca. 74, 57, and 50 kilodaltons (K) were detected (Fig. 1, lane a), whereas two major polypeptides of about 55 and 50 K were detected in cell extracts infected with HV 76-118 (Fig. 1, lane d), when immunoprecipitated with the same immune sera. On the other hand, antiserum against HV 76-118 immunoprecipitated two polypeptides with molecular weights of 55 and 50 K in extracts from cells infected with HV 76-118 (Fig. 1, lane e) and reacted with one major polypeptide of 50 K in cells infected with HFRS virus B-1 (Fig. 1, lane b).

When infected cells were labeled with [3H]mannose, the antiserum against HFRS virus B-1 reacted with a major polypeptide of 57 K and a minor one of 74 K from cell extracts infected with HFRS virus B-1 (Fig. 1, lane g), and the same sera precipitated one polypeptide of 55 K from cell extracts infected with HV 76-118 (Fig. 1, lane j). On the other hand, antiserum against HV 76-118 reacted with a major polypeptide of 55 K in extracts from cells infected with strain 76-118 (Fig. 1, lane k) and did not precipitate any polypeptides in cell extracts infected with HFRS virus B-1 (Fig. 1, lane h). These data indicate that HV 76-118 induces a major 50-K polypeptide and a minor glycosylated one of 55 K. On the other hand, HFRS virus B-1 induces three major polypeptides with molecular weights of 74,000, 57,000, and 50,000, the former two polypeptides being glycosylated. By the immunoprecipitation experiments, polypeptides of both strains of virus were shown to be immunologically related.

**Immunological examination of HFRS virus B-1 and HV 76-118 strains.** Immunological differences between HV 76-118 and HFRS virus B-1 strains were examined by IF and NT tests with four sera from rats (two sera each from rats infected with HV 76-118 and HFRS virus B-1 strains) and four human sera from HFRS patients found in Osaka.

In the IF test, rat sera immunized with HV 76-118 had the same titer against both strains, but antiserum against HFRS virus B-1 showed higher titers (four times) to HFRS virus B-1 than to HV 76-118. When these sera were examined by the NT test, a remarkable difference was observed: immune rat

<p>| TABLE 1. Comparison of antigenicity between HV 76-118 and HFRS virus B-1 strains by IF and NT tests |
|--------------------------------------------------|--------------------------------|-------------------------------|</p>
<table>
<thead>
<tr>
<th>Origin of serum</th>
<th>IF titer against a)</th>
<th>HFRS virus B-1</th>
<th>NT titer against b)</th>
<th>HFRS virus B-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (HV 76-118)</td>
<td>4,096</td>
<td>4,096</td>
<td>1,280</td>
<td>80</td>
</tr>
<tr>
<td>2 (HV 76-118)</td>
<td>1,024</td>
<td>1,024</td>
<td>80</td>
<td>20</td>
</tr>
<tr>
<td>3 (HFRS virus B-1)</td>
<td>16,000</td>
<td>64,000</td>
<td>160</td>
<td>2,560</td>
</tr>
<tr>
<td>4 (HFRS virus B-1)</td>
<td>16,000</td>
<td>64,000</td>
<td>320</td>
<td>5,120</td>
</tr>
<tr>
<td>Human</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>16,000</td>
<td>32,000</td>
<td>320</td>
<td>2,560</td>
</tr>
<tr>
<td>2</td>
<td>1,600</td>
<td>3,200</td>
<td>64</td>
<td>256</td>
</tr>
<tr>
<td>3</td>
<td>1,024</td>
<td>4,096</td>
<td>128</td>
<td>256</td>
</tr>
<tr>
<td>4</td>
<td>4,096</td>
<td>16,000</td>
<td>128</td>
<td>256</td>
</tr>
</tbody>
</table>

a) Antibody titers are expressed as the reciprocal of the highest serum dilution giving specific fluorescence.

b) Antibody titers are expressed as the reciprocal of the highest serum dilution giving a 50% reduction in focus formation.
sera against one strain showed a higher titer (4 to 16 times) to
the homologous strain than to the heterologous strain. When
human sera were also tested by the IF and NT tests, all sera
showed higher titers (ca. two to four times in the IF test and
two to eight times in the NT test) against HFRS virus B-1
than against HV 76-118 (Table 1).

Isolation and characterization of hybrid cell lines. In 10 trial
fusions, about 2,500 hybrid cell lines were obtained. Culture
fluids from the microtiter wells containing an individual
colony were screened for antibodies against HV by the
indirect IF test with HV-infected cells. By those screening
tests, 39 clones were shown to secrete detectable antibodies.
Of these, 10 clones were found to react with cellular anti-
gens. Before further characterization, the other 29 clones
producing antibodies against HV antigen were cloned twice
by the limiting-dilution method to ensure stability and to
eliminate the possibility of contamination with other clones.
A total of 9 hybridomas could not be established stably
during this process, and 20 hybridomas were finally used for
experiments.

Further characterization was attempted by using the cul-
ture fluid after the last cloning. Clones could be divided into
three groups depending on the staining pattern by the IF test.
Group I was characterized by an immunofluorescent pattern
of discrete patches in the cytoplasm of infected cells, group
II reacted with antigens in large granular dots in the cyto-
plasma, and group III showed fine granular dots throughout
the cytoplasm (Fig. 2, a to c). All antibodies belonging to
group I showed granular dots of IF staining on the membrane
of HV-infected cells (Fig. 2d). All 20 clones were sub-
sequently injected into mice for the production of ascitic fluid.

Mice injected with all clones except one (clone 26B) pro-
duced ascites in 10 to 14 days after inoculation with hybri-
doma cells.

Specificities of viral polypeptides detected by monoclonal
antibodies. Vero E6 cells were infected with HV 76-118 and
HFRS virus B-1, then labeled with [35S]methionine, and
immunoprecipitated with monoclonal antibodies as de-
scribed above. Clones 40A and 43B and clones 33B and 80A
were chosen as the representatives of group I and group III,
respectively, and the patterns of SDS-PAGE obtained after
immunoprecipitation are shown in Fig. 3. When monoclonal
antibodies were first assayed against the extract of HV 76-
118-infected cells, all monoclonal antibodies of group I
reacted specifically with a polypeptide of 55 K (Fig. 3, lanes
a and d), and those of group III precipitated with a polypep-
tide of 50 K (Fig. 3, lanes m and p). However, antibodies of
group II did not immunoprecipitate any polypeptides. Then,
the same antibodies of group I were assayed with the extract
of HFRS virus B-1-infected cells. Three of five clones
reacted with a polypeptide of 57 K (Fig. 3, lane e; Table 2),
but antibodies from the other two clones (40A and 176E) did
not precipitate any polypeptides (Fig. 3, lane b; Table 2). For
antibodies from group III, 10 of 13 clones precipitated a 50-K
polypeptide (Table 2; Fig. 3, lanes n and q).

Those antibodies were further characterized by the im-
munoprecipitation with [3H]mannose-labeled cell extracts.
Monoclonal antibodies of group I precipitated a 55-K polypep-
dide from cell extracts infected with HV 76-118 virus and
a 57-K polypeptide in cell extracts infected with HFRS virus
B-1 (Fig. 3, lanes g to l). Antibodies from clones of groups II
and III did not react with any polypeptides labeled with

![FIG. 2. Fluorescence photomicrographs of virus-specific antigens in Vero E6 cells infected with HV (strain 76-118). Antigens stained by
group I (a), group II (b), and group III (c) antibodies were found in the cytoplasm. Group I antibody-stained antigens in the membranes of in-
fected cells are shown in (d).](http://jvi.asm.org)
[3H]mannose. This finding suggests that the 55-K polypeptide of HV 76-118 and the 57-K polypeptide of HFRS virus B-1 are glycosylated.

**Reactivity patterns of monoclonal antibodies in IF and NT tests.** All monoclonal antibodies were examined in IF and NT tests. Only antibodies from group I had neutralizing activity, and based on this, hybridoma clones were classified into three subgroups, designated for convenience as I-A, I-B, and I-C (Table 2). Group I-A monoclonal antibodies had considerably high NT titers as well as IF titers against the homologous virus (HV 76-118) but showed little reactivity with HFRS virus B-1 in NT and IF tests. Group I-B

### Table 2. Monoclonal antibodies against HV

<table>
<thead>
<tr>
<th>IF pattern and group</th>
<th>Hybridoma clone</th>
<th>Antibody reactivity to HV</th>
<th>Antibody reactivity to B-1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IF titer (x10^3)</td>
<td>NT titer (fold)</td>
</tr>
<tr>
<td>Discrete patch</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-A</td>
<td>40A</td>
<td>64</td>
<td>600</td>
</tr>
<tr>
<td></td>
<td>176E</td>
<td>64</td>
<td>600</td>
</tr>
<tr>
<td>I-B</td>
<td>43B</td>
<td>64</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>99B</td>
<td>4</td>
<td>40</td>
</tr>
<tr>
<td>I-C</td>
<td>21B</td>
<td>64</td>
<td>&lt;20</td>
</tr>
<tr>
<td>Large granular dots</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II-A</td>
<td>26B</td>
<td>4</td>
<td>&lt;20</td>
</tr>
<tr>
<td>II-B</td>
<td>133E</td>
<td>64</td>
<td>&lt;20</td>
</tr>
<tr>
<td>Fine granular dots</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III-A</td>
<td>149A</td>
<td>16</td>
<td>&lt;20</td>
</tr>
<tr>
<td></td>
<td>19B</td>
<td>256</td>
<td>&lt;20</td>
</tr>
<tr>
<td></td>
<td>33B</td>
<td>64</td>
<td>&lt;20</td>
</tr>
<tr>
<td>III-B</td>
<td>43A</td>
<td>16</td>
<td>&lt;20</td>
</tr>
<tr>
<td></td>
<td>80A</td>
<td>64</td>
<td>&lt;20</td>
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<tr>
<td></td>
<td>85A</td>
<td>64</td>
<td>&lt;20</td>
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<tr>
<td></td>
<td>90A</td>
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<td>&lt;20</td>
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<tr>
<td></td>
<td>92A</td>
<td>4</td>
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<tr>
<td></td>
<td>4B</td>
<td>16</td>
<td>&lt;20</td>
</tr>
<tr>
<td></td>
<td>14B</td>
<td>256</td>
<td>&lt;20</td>
</tr>
<tr>
<td></td>
<td>83B</td>
<td>256</td>
<td>&lt;20</td>
</tr>
<tr>
<td></td>
<td>141D</td>
<td>256</td>
<td>&lt;20</td>
</tr>
<tr>
<td></td>
<td>255D</td>
<td>256</td>
<td>&lt;20</td>
</tr>
</tbody>
</table>

* MA, Reaction with membrane antigen of infected Vero E6 cells. +, Positive; −, negative.
* The molecular weight of the polypeptide found by SDS-PAGE is measured in kilodaltons.
A monoclonal antibodies reacted with both strains of viruses in the IF test, but they had lower NT activity against both strains when compared with that of group I-A. The antibodies classified as I-C had high titers in IF tests against both strains but little neutralizing activity.

Of 20 clones, only 2 clones belonged to group II. Antibodies from one of these (26B) reacted only with HV antibodies in the IF test; the other hybridoma had raised IF titers against both strains. These monoclonal antibodies neither immunoprecipitated any polypeptide nor had neutralizing activity.

Monoclonal antibodies belonging to group III were shown to have high titers in the IF assay but did not have neutralizing activity. They were also classified into two subgroups (groups III-A and III-B). Subgroup III-A antibodies reacted only with HV antigens in the IF test, but III-B antibodies reacted with both strains in the IF test and in SDS-PAGE.

**DISCUSSION**

HV 76-118 was reported to be closely related with other viruses that cause HFRS from the immunological point of view (2, 3, 6, 12, 14, 15, 16, 22, 24), and according to morphological evidence obtained by electron microscopy (18, 25) and RNA analysis (20), it belongs in the family Bunyaviridae. In general, since bunyaviruses contain at least three structural proteins including two glycoproteins designated G1 and G2 (1), viruses that cause HFRS are expected to be composed of the same structural components. In our study, two polypeptides in extracts of cells infected with HV 76-118 and three polypeptides in extracts of cells infected with HFRS virus B-1 were identified by using antisera; two of the three (74- and 57-K) polypeptides of HFRS virus B-1 were glycosylated. On the other hand, only two polypeptides were detected with antisera in cells infected with HV 76-118, and one polypeptide (55 K) was glycosylated. Although it is not certain whether another glycoprotein exists in cells infected with HV 76-118, it may be speculated that another polypeptide was undetectable in the HV 76-118-infected cells because of small amounts of antigen.

To study them in more detail, we then tried to isolate monoclonal antibodies against HV, and 20 hybridomas were isolated. These antibodies were classified into three groups on the basis of the IF pattern of the HV-infected cells. Moreover, monoclonal antibodies belonging to two different groups immunoprecipitated two different polypeptides; i.e., monoclonal antibodies of group I, which showed a pattern of discrete patches in the cytoplasm of infected cells precipitated with a 55-K glycoprotein in the IF test reacted with membrane antigens in 76-118 HV-infected cells and had neutralizing activity against HV. This evidence suggests that the 55-K polypeptide may apparently exist in the envelope of viral particles and also apparently localize to the cell surface of infected cells. Furthermore, if viruses that cause HFRS belong to bunyaviruses as described elsewhere (18, 20, 25), the progeny virus particles probably assemble and bud into the Golgi cisternae. The IF pattern detected by monoclonal antibodies may show the localization of HFRS virus particles in infected cells (Fig. 2). When extracts of cells infected with HFRS virus B-1 were immunoprecipitated with these monoclonal antibodies, a glycoprotein having a molecular weight of ca. 57,000 was detected. However, some clones did not react with extracts of HFRS virus B-1-infected cells. It may be thought that this 57-K glycoprotein is antigenically closely related with the 55-K polypeptide of HV and that at least three antigenic determinants exist in the envelope glycoprotein of HV, one of which may be strain specific and other two of which may be strain common. Next, since two glycoproteins (57 and 74 K) were detected in cells infected with HFRS virus B-1 as described above, it may be thought that one of them is a precursor protein. However, monoclonal antibodies belonging to group I precipitated one glycoprotein, and consequently, it was confirmed that two glycoproteins with different molecular weights exist in cells infected with HFRS virus B-1.

All monoclonal antibodies from 13 hybridomas which showed a pattern of fine dots in the cytoplasm by the IF test and reacted with 50-K polypeptides had no neutralizing activity. Recently, it has been reported that a 50-K polypeptide was detected in the virion of HV 76-118 and is supposed to exist in its nucleocapsid (20). These monoclonal antibodies of group III may apparently react with the polypeptide in the nucleocapsid. Again, there are strain-specific and strain-common epitopes in the 50-K polypeptide, as is the case with the 55-K glycoprotein.

Since the monoclonal antibodies that showed large granular dots in the cytoplasm did not react with any polypeptide in HV- or B-1 strain virus-infected cells and had no neutralizing activity either, the kind of polypeptides with which those antibodies could react is not clear. This finding may be explained as follows: (i) the solubility of the antigens may have been incomplete with our method, (ii) the antigenicity of the polypeptides may have been changed by destruction of the three dimensional structure during detergent treatment, and (iii) since affinity between an antigen and an antibody can be very weak, the antibody may have been readily detached.

Recently, monoclonal antibodies for HV were isolated and antigenic analyses of HFRS viruses were attempted (4). In their report, Franko et al. (4) also classified the monoclonal antibodies into three groups by IF patterns, and only a 50-K polypeptide was detected by these monoclonal antibodies. It is not certain whether our manifestation in the IF test would correspond to their results, but group I monoclonal antibodies reacted with a 55-K glycoprotein, and group III monoclonal antibodies immunoprecipitated a 50-K polypeptide in our results.

Although HFRS B-1 was immunologically related to HV, a remarkable difference between them was observed in NT tests with specific immune sera prepared in rats, and this fact has also been confirmed by using monoclonal antibodies of group I. This finding suggests that major antigenic differences between the two strains exist in the envelope proteins which usually exert a main role in virus neutralization. HFRS virus in Japan has been recognized to be transmitted from wild or experimental rats, and the symptoms of this disease have been generally mild, whereas that in Korea has been transmitted from Apodemus agrarius coreae, and the symptoms appear to be comparatively severe. It is at present not clear whether the above virological and immunological differences between the two strains is related to this difference in disease severity in the two countries. This question will be elucidated by examining more strains isolated in the two countries.

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