Comparison of the Pathogenicity of Rinderpest Virus in Different Strains of Rabbits

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ABSTRACT. Pathogenicity of the lapinized Nakamura-III (L) strain of rinderpest virus (RPV) was examined in four strains of rabbits consisting of two inbred strains (NW-NIBS and DUY-NIBS) and two outbred strains maintained in closed colony (NW-NIBS and JW-NIBS) using a marmoset lymphoblastoid cell line, B95a cell-passage virus and tissue homogenates of virus-infected rabbits. The cell culture virus was found to maintain virulence for rabbits of both closed colony and inbred NW-NIBS strain similar to the homogenate virus. Among the strains investigated, inbred NW-NIBS strain showed the highest susceptibility to RPV. Thus experimental model in an inbred rabbit using cell culture virus became useful.—KEY WORDS: inbred strain of rabbit, pathogenicity, rinderpest virus.


Rinderpest virus (RPV), belonging to the morbillivirus subgroup of paramyxoviridae causes a fatal disease in cattle. The lapinized Nakamura-III (L) strain of RPV [10] has been extensively used as a model for the pathogenesis of RPV. In rabbits infected with the L strain, leukopenia, severe lymphoid necrosis, immunosuppression, and transient production of autoantibodies are induced [1–4, 6, 7, 13, 14]. Thus this model is considered to be useful to investigate the immunological disturbance caused by virus infection. However, this animal model has two drawbacks. Firstly, the rabbits are of outbred, so that the study of pathogenesis under the homogeneous genetic background is impossible. Secondly, the virus inoculum consists of the homogenate of the virus-infected lymphoid tissues, so that detailed virological approaches such as virus titration are difficult. Although the homogenate virus can be passed in Vero cells, it loses virulence for rabbits rapidly by passages [4, 5]. Two major progress appeared to solve these drawbacks, i.e. the development of inbred strains of rabbits and the finding that the L strain grown in B95a cells, a marmoset lymphoblastoid cell line [8], maintain virulence for rabbits. By using B95a cell, it is also possible to titrate infectivity of the homogenate virus [9].

MATERIALS AND METHODS

Cells and virus: B95a cells, derived from B95-8 cell line, a marmoset B lymphoblastoid cell transformed by Epstein-Barr virus [9], were propagated in RPMI 1640 (Nissui, Tokyo) supplemented with 10% fetal bovine serum under a 5% CO2-contained humidified atmosphere. The maintenance medium for B95a cells was RPMI 1640 supplemented with 2.5% fetal bovine serum.

Homogenate virus was prepared from the mesenteric lymph nodes of the rabbits infected with the L strain as reported previously [13]. Cell culture virus consisted of supernatant of sonicated B95a cells which were co-cultured with the homogenate virus. These cells were sonicated without further passages. The median tissue culture infectious dose (TCID50)/ml of the homogenate virus and the cell culture virus titrated in B95a cells were 103.9 and 104.6, respectively.

Animals: Rabbits used in this study were developed in the Nippon Institute for Biological Science, Tokyo, Japan. They consisted of two inbred strains (NW-NIBS and DUY-NIBS) and two outbred strains maintained in closed colony (NW-NIBS and JW-NIBS). Two experiments were conducted. For the comparison of the virulence between two virus sources mentioned above, 1 ml of both viruses were inoculated intravenously to one rabbit of inbred NW-NIBS strain and one of outbred NW-NIBS strain. To compare the sensitivity to RPV-L strain among rabbit strains (inbred NW-NIBS and DUY-NIBS, and outbred JW-NIBS), cell culture virus was inoculated intravenously to one rabbit of each strain.

Clinical investigations: Rectal temperature was recorded daily. Total white blood cell (WBC) numbers in the peripheral blood were counted by routine method (Unopette Test 58.56; Becton-Dickinson, NJ, U.S.A.). Lymphocyte numbers were calculated by the total WBC numbers multiplied by the percentage of lymphocytes in the blood smear.

Virological and histopathological examinations: The rabbits were sacrificed at 4 days post inoculation (dpi), under anesthesia and the lymphoid tissues consisting of the spleen, Peyer's patches, mesenteric lymph nodes, and appendixes were collected and weighed. Virus infectivity titer in 10% homogenate (w/v) of these tissues was titrated in B95a cells and expressed as TCID50/ml.

For histopathological examinations, the tissues were fixed by neutral buffered formalin, embedded in paraffin,
sectioned at 2 μm of thickness, and stained with hematoxylin-eosin (H-E). For immunohistochemical examinations, ordinary avidin-biotin peroxidase complex (ABC) method [12] was used. Briefly, after incubated with 0.3% H₂O₂/methanol and normal goat serum, deparaffinized sections were incubated with 1:1000-diluted monoclonal antibody against RPV nucleocapsid protein (NP) [11] at 4°C for overnight. Then incubated with biotinylated second antibody at 37°C for 40 min, the sections were subjected for the Vectastain peroxidase method (Vector Laboratories Inc., CA, U.S.A.) using 3,3'-diaminobenzidine-tetrahydrochloride (Sigma Chemical Co., MO, U.S.A.) as the chromogen. After the ABC staining, the sections were counterstained with hematoxylin. In negative controls, 1:200-diluted normal mouse serum was used as the primary antibody.

RESULTS

Comparison of the pathogenicity between homogenate virus and cell culture virus: Clinically, both viruses showed similar pathogenicity both in inbred and closed colony NW-NIBS strains, such as transient fever higher than 40°C at 3 dpi, marked lymphopenia associated with decreased lymphocytes (data not shown). Histological changes and virus growth in the lymphoid tissues were examined at 4 dpi, and the results were summarized in Table 1. Remarkably high virus titers were detected in the gut-associated lymphoid tissues such as the Peyer’s patches, mesenteric lymph nodes, and at relatively low level in the spleen. The histopathological changes were also similar between the homogenate virus and the cell culture virus both in inbred and outbred NW-NIBS rabbits (data not shown).

Table 1. Virulence of the cell culture virus and homogenate virus in an inbred NW-NIBS strain

<table>
<thead>
<tr>
<th>Organ</th>
<th>Cell culture virus (10⁶TCID₅₀)</th>
<th>Homogenate virus (10⁶⁹TCID₅₀)</th>
<th>Virus growth¹</th>
<th>Lymphoid depletion</th>
<th>Lymphoid necrosis</th>
<th>Virus antigen²</th>
<th>Virus growth³</th>
<th>Lymphoid depletion</th>
<th>Lymphoid necrosis</th>
<th>Virus antigen</th>
<th>Virus growth³</th>
</tr>
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<tr>
<td>Appendix</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>10⁴.¹</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>10³.⁹</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peyer’s patches</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>10⁵.⁰</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>10⁴.⁶</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mesenteric LN³</td>
<td>ND¹</td>
<td>ND¹</td>
<td>ND</td>
<td>10⁴.⁸</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>10³.⁸</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superficial LN</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>ND</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>10².¹</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Bone marrow</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>ND</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

+++; Marked. ++; Moderate. +; Slight. −; Negative.

¹ Organ samples were collected at 4 days post inoculation.

² Virus antigen detected by ABC method.

³ TCID₅₀/ml

⁴ Lymph nodes

⁵ Not determined.

Fig. 1. Appendix of the inbred NW-NIBS rabbit infected with the cell culture virus (a). Necrosis of the lymphoid follicle is severe (b) and depletion of lymphocytes in thymus-dependent area (c) is also observed. a-c; H-E staining, a, × 25 b, × 50, c, × 100.
shown). Almost all the lymphoid tissues examined had lymphocyte depletion and lymphoid necrosis associated with nuclear debris of lymphocytes and phagocytic reaction of macrophages, which are characteristic to the infection of this virus [1, 10, 13]. The most severe lesions were observed in the appendices and Peyer’s patches. In these two tissues, depletion of lymphocytes was found in the thymus-dependent area and severe necrosis in the center of the follicles (Fig. 1). In the other lymphoid tissues, lymphocyte depletion was also observed, but the necrosis of lymphoid follicles was milder.

Positive ABC stain indicated a marked accumulation of virus antigens in the appendices, Peyer’s patches and the other lymphoid tissues (Fig. 2). The virus antigens were mainly detected in lymphocytes and activated macrophages in the lymphoid follicles. Thus, both inbred and outbred strains of rabbits reacted similarly to the homogenate virus and the cell culture virus in clinical, virological and histological aspects.

Comparison of the host susceptibility to the L strain among three strains of rabbits: In a subsequent experiment, susceptibility of the two inbred DUY-NIBS and NW-NIBS strains and one outbred JW-NIBS strain to the cell culture virus was examined. All the rabbits developed similarly fever higher than 40°C at 3 dpi. As shown in Table 2, the two inbred strains developed a marked leukopenia and lymphopenia, whereas they were not so remarkable in outbred JW-NIBS strain. The highest virus yield in the lymphoid tissues was observed in an inbred NW-NIBS strain. All the four strains of rabbits showed histopathologically the similar changes by H-E staining. By immunohistochemical examination, the amount of virus antigens seemed to be more abundant in the appendix of an inbred NW-NIBS strain and of outbred JW-NIBS strain than an inbred DUY-NIBS strain (Fig. 3).

DISCUSSION

In the present study, the cell culture virus prepared in the B95a cells was shown to have virulence similar to the homogenate virus. Therefore, the use of the cell culture virus is expected to facilitate the virological study on pathogenesis of RPV [9]. Two inbred strains of rabbits were found to be susceptible to the infection of the cell culture virus. Transient high fever, lymphopenia and histopathological changes in the lymphoid tissues, which were characteristics of RPV infection in outbred rabbits [1, 10, 13], were similarly observed in the inbred strains. Therefore, the use of B95a-passaged L strain and inbred strains of rabbits will provide a useful model for detailed investigation of RPV pathogenesis in both virological and immunological aspects. Distribution of viral antigen appeared to be slightly different among the rabbit strains tested. The virus-induced lymphopenia was found to be more marked in two inbred NW-NIBS and DUY-NIBS strains than the outbred JW-NIBS strain. The virus growth in the lymphoid tissues was markedly high in the inbred NW-NIBS strain. Therefore, the inbred NW-NIBS

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>No. of WBC in dpia)</th>
<th>Virus titerb) in</th>
<th>Appendix</th>
<th>Peyer’s patches</th>
<th>Mesenteric lymph nodes</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>10^4 (c)</td>
<td>10^0</td>
</tr>
<tr>
<td>Inbred NW-NIBS</td>
<td>10500</td>
<td>9700</td>
<td>4200</td>
<td>1400</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inbred DUY-NIBS</td>
<td>5600</td>
<td>5400</td>
<td>2700</td>
<td>3700</td>
<td>10^2-4</td>
<td>10^1</td>
</tr>
<tr>
<td>Outbred JW-NIBS</td>
<td>4900</td>
<td>7800</td>
<td>3100</td>
<td>3500</td>
<td>10^2-2</td>
<td>ND</td>
</tr>
</tbody>
</table>

a) Days post inoculation  
b) Virus titer at 4 days post inoculation  
c) TCID_50/ml  
d) Not detected
strain appears to be most susceptible to RPV-L strain infection. However, the reproducibility remains to be investigated due to the limited numbers of inbred rabbits available at the moment.

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REFERENCES