Pathogenesis of Rinderpest Virus Infection in Rabbits

I. Clinical Signs, Immune Response, Histological Changes, and Virus Growth Patterns

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Rabbits were intravenously inoculated with an attenuated rinderpest virus (L strain), and general patterns of the disease were investigated. The rabbits developed fever with concomitant occurrence of diarrhea and lymphopenia. Early production of interferon was followed by a rise of neutralizing antibody. Histological examinations revealed an involvement of all of the lymphoid tissues, with primary lesions consisting of necrosis of the lymphoid follicles and formation of giant cells. Immunofluorescent examinations suggested that the virus growth was present in almost all of the lymphoid tissues. The possibility of application of this experimental system for the study of systemic infection by measles virus was discussed.

Measles virus offers interesting problems about the relationship between pathogenesis and host immune mechanism in systemic virus diseases. However, the present experimental conditions using nonhuman primates, which are the only laboratory animals susceptible to measles virus, in the study of pathogenesis of measles virus infection are not satisfactory, mostly because of a high frequency of natural infection with measles virus (17) and relative difficulty in application of immunological approaches compared with other laboratory animals such as mice, guinea pigs, and rabbits.

Therefore, we examined the possibility of using rinderpest virus instead of measles virus, since these two viruses and canine distemper virus belong to the same medipest subgroup of paramyxoviruses, and they are all known to produce essentially similar types of diseases in their respective natural hosts (Table 1).

Among several strains of rinderpest virus, the L strain has been attenuated by long serial passages in rabbits and can be safely handled in the laboratory. The L strain was shown to have high virulence in rabbits, producing clinical signs generally similar to those found in cattle infected with the virulent virus (3).

In the present study, the general course of rinderpest virus infection in rabbits, including clinical signs, immune response, virus growth patterns, and histopathological changes, was

investigated, and the possibility of using this experimental system as a model for the study of systemic virus diseases was examined.

MATERIALS AND METHODS

Viruses. The lapinized Nakamura III strain (L strain) (10) was used for rabbit inoculation. The mesenteric lymph nodes of the rabbits were collected 5 days after intravenous inoculation of the virus, made into 10% tissue extract, and stored at -80°C as a stock virus. This stock virus had a titer of $10^{3.0}$ mean infective dose per ml in rabbits, and 1 ml of the undiluted virus was intravenously inoculated in infection experiments.

The LA strain, which was further attenuated by passage of the L strain in chick embryos, was adapted to grow in Vero cell cultures (12), and the infected Vero cell culture fluid was used as a stock of challenge virus for neutralization tests.

Rabbits. Albino rabbits of both sexes, JW-NIBS strain (average body weight of 1.5 kg), were obtained from the closed colony of the Nippon Institute for Biological Science (Tachikawa, Tokyo) at the age of 3 months.

Histological examination. Various lymphoid tissues and other organs removed at autopsy were fixed in 10% Formalin, dehydrated, and embedded in paraffin as usual. Thin sections were stained by hematoxylin and eosin. Histological lesions were graded as +++, ++, +, or -, depending on the distribution and intensity of pathological changes. Scores of 3, 2, 1, and 0 were given to +++, ++, +, and -, respectively.

TABLE 1. Comparison of pathogenicity of measles, canine distemper, and rinderpest viruses^a

Virus	Latent period (days)	Clinical signs							Histopathological lesion			
		Fever	Respiratory signs	Conjunctivitis	Exanthema	Enanthema	Gastro-intestinal signs	Giant cell pneumonia	Encephalitis	Giant cell	Inclusion bodies	Lymphoid necrosis
Measles (man)	10-12	Biphasic	+	+	+	+	+	±	+	<0.1%	+	-
Canine distemper (dog)	3-6	Biphasic	+	+	+	+	+	+	+	10-30%	+	+
Rinderpest (cattle)	6-9	Monophasic	+	+	±	+	+	+	?	Rare	+	+

^a Arranged from the original table made by Imagawa (4), with several modifications to show the general similarities in the pathogenicity of the three viruses in their respective natural hosts. Virus transmission occurs by the respiratory route, and immunity persists life long.

Immunofluorescent staining. Anti-rinderpest serum was collected from a rabbit inoculated intravenously with the LA strain 4 weeks earlier. The antiserum was fractionated by 50% saturated ammonium sulfate, and its globulin fraction was conjugated with fluorescein isothiocyanate. The staining titer of the conjugated serum was 128, and its fluorescein/protein ratio was 1.8. Before the experiments, specificity of the conjugated serum was confirmed by specific blocking of the fluorescence by cattle anti-rinderpest serum on the virus-infected Vero cells.

The tissues were rapidly frozen in hexane chilled in dry ice-acetone, cut by cryostat to 4-μm thickness, and fixed in acetone at -20 C for 20 min. Staining was made at 4 C overnight. The degree of virus-specific fluorescence was recorded as +++, ++, +, or -, depending on its distribution and intensity, and these recordings were converted into scores of 3 to 0, as in the histological examination.

Detection of viremia. Since the L strain of rinderpest virus does not regularly produce a cytopathic effect in tissue culture (5), detection of viremia was conducted by immunofluorescent staining of peripheral leukocytes. Heparinized blood was collected from the ear veins at 1, 2, 3, 4, 5, 7, 10, and 14 days after virus inoculation, and the buffy-coat layer was separated by gravitational sedimentation at 37 C for 50 min in the presence of 1% dextran. After being washed with phosphate-buffered saline, the leukocyte-rich fraction was placed on a slide glass, air-dried, fixed in acetone, and stained by fluorescent antibody.

Virus neutralization test. Neutralizing antibody was titrated in tubes of Vero cell cultures as described previously (12). Antibody titer was expressed as the reciprocal of the serum dilution that protected 50% of the tubes from viral cytopathic effect.

Interferon (IF) titration. Induction of IF into the sera of rabbits after virus infection was examined by the plaque reduction method in RK 13 cells by using vesicular stomatitis virus as a challenge virus. Titer was expressed as the reciprocal of the serum dilution that resulted in 50% reduction of the plaque numbers.

RESULTS

Clinical signs. Fever and diarrhea were observed as the main symptoms characteristic of rinderpest virus infection in rabbits. Intravenous inoculation of the virus consistently induced transient fever higher than 40 C on the 2nd to 3rd days of infection. Generally the temperature returned to normal degree within 2 to 3 days later. Severe diarrhea was also observed on days 2 to 5 in most of the infected rabbits. All of the infected rabbits recovered quickly and remained apparently healthy during the observation periods for more than 3 months.

Hematological examination. Severe leukopenia was induced within 2 days after infection and persisted for over 6 weeks (Fig. 1). Differential counting disclosed a marked decrease in the

FIG. 1. Hematological examination of rabbits infected with rinderpest virus. Symbols: R131 through R133, uninoculated control; R134 through R136, inoculated group.

number of lymphocytes. There was no significant change in numbers of pseudoeosinophils, basophils, eosinophils, and monocytes. Therefore, the lymphopenia was considered a main cause of the leukopenia.

Production of IF and neutralizing antibody in serum. The results of IF production and neutralizing antibody are shown in Fig. 2. IF was induced in the sera on day 4, with a maximal titer of 2^8 on days 4 to 6, and disappeared on the 8th day. At the time of clearance of IF, development of neutralizing antibody was observed.

Viremia. Three rabbits were intravenously inoculated with the virus. At 2 days after inoculation, few virus antigen-positive cells were detected. At 3 to 5 days, most of the nucleated cells having a large cytoplasm contained virus antigens. At 7 days, virus antigen-positive cells were almost completely eliminated from the peripheral blood. Thus, the viremia was suggested to occur between 2 to 5 days after infection.

Histopathology. A total of 40 rabbits were inoculated with the virus, and five to six rabbits were killed at each sampling at 2, 3, 4, 5, 7, 10, and 14 days after infection. Since a primary target of rinderpest virus in cattle and rabbits

FIG. 2. Development of IF and virus neutralizing antibody.

FIG. 3. Time courses of virus growth and histological changes in the lymphoid tissues. The grade of virus growth and histological change was taken as the average of the scores in each sampling group consisting of five to six rabbits.

has been generally thought to be the lymphoid tissues (3, 14), special attention was paid to the histological changes in the lymphoid tissues. The most typical lesions in the lymphoid tissues were giant cell formation and necrosis of the lymphoid follicles. Therefore, the time course of the histological changes was roughly depicted in Fig. 3 by using giant cell formation and lymphoid necrosis as indicators. For comparison, the time course of virus growth was also shown by using virus-specific fluorescence as an indicator.

At 2 to 5 days after infection, the giant cell formation was observed frequently in the superficial lymph nodes, including submandibular, axillary, inguinal, and popliteal lymph nodes, spleen and thymus, and was less frequently observed in the mesenteric lymph nodes, tonsils, and gut-associated lymphoid tissues (GALT) such as Peyer's patches, appendix, sacculus rotundus, and cecal tonsil (Fig. 4, 5). In these lymphoid tissues, the giant cells were localized mainly in the parenchyma, including the germinal center, in a pattern similar to

FIG. 4. Giant cells located in the parenchyma of the submandibular lymph node; taken 3 days after infection. $\times 100$.

those observed in measles virus-infected monkeys (16). Most of the giant cells were of reticular type similar to those observed in the measles virus-infected monkeys. A number of large cells with foamy cytoplasm were localized around the giant cells. After giant cell forma-

FIG. 5. *High-magnification view of the giant cells shown in Fig. 4. The giant cells are characteristically composed of somewhat radially or irregularly arranged nuclei which contain fine chromatin, a small nucleolus, and lightly stained, large eosinophilic cytoplasm. Small round lymphoid cells are present around the giant cells in large numbers. $\times 400$.*

tion, a marked necrosis of the lymphoid follicles involving both the lymphoid and reticular cell elements was revealed generally on days 3 to 5 (Fig. 6). In contrast to the distribution of giant cells, lymphoid necrosis was most conspicuous in the mesenteric lymph nodes and GALT. On day 10, the above-mentioned lesions were completely eliminated and were replaced by granular tissue in which no lymphoid cells were seen even on day 14.

Virus growth. Immunofluorescent staining was conducted with the thymus, tonsil, spleen, axillary, inguinal, popliteal, and mesenteric lymph nodes, appendix, Peyer's patches, bone marrow, liver, and kidney. The general time course of virus growth is shown in Fig. 3. There was a good correlation between the time course of histological changes and that of virus growth. At 2 to 3 days after infection, virus-specific fluorescence was first detected in the cytoplasm of the lymphoreticular cells in the lymphoid follicles in most of the lymphoid tissues. Virus growth in the lymphoid tissues reached maximum on days 5 to 7. During this period, virus antigen was present in the thymus, tonsil, spleen, various superficial lymph nodes, mesenteric lymph nodes, appendix, and Peyer's patches (Fig. 7, 8). In these organs, localization of virus antigen was as follows. Both the cortex and medulla of the thymus were involved, showing a large amount of virus antigen in a disseminated pattern. The tonsil was involved in both the lymphoid tissues and epithelium, the main lesion being present in the former and less apparent in the latter. The spleen was widely affected, the virus antigen being demon-

strated in almost the entire area of the white pulp and in the sinusoid lining of the red pulp. A particularly large amount of virus antigen was detected in the germinal center in the white pulp. The superficial and mesenteric lymph nodes were similarly affected; the parenchyma was entirely filled with virus antigen, with a very large amount appearing in the mesenteric lymph nodes. The Peyer's patches and appendix also contained a large amount of virus antigen in the lamina propria.

Virus antigen was not detected in the bone marrow and kidney in any of the rabbits examined. In the liver, virus antigen was present in the epithelial cells lining the bile ducts in some rabbits on day 7. Specific fluorescence was absent in Kupffer cells in the liver.

Thus, virus growth was thought to occur in the lymphoid tissues as a main target. Among them, virus growth appeared to be most efficient in the mesenteric lymph nodes, Peyer's patches, and appendix.

After day 7, the virus antigen was eliminated quickly from the lymphoreticular cells. On the other hand, a small amount of virus antigen

FIG. 6. *Necrotic lesion in Peyer's patch of the small intestine; taken 4 days after infection. $\times 100$.*

FIG. 7. *Thymus lobes filled with intense fluorescence; taken 7 days after infection. $\times 100$.*

FIG. 8. Virus antigen widely disseminated in the lymphoid follicle of the spleen; taken 7 days after infection. Note the presence of virus antigen in large amount surrounding the central arteriole. $\times 100$.

appeared in the epithelium in the small intestine and appendix, suggesting virus growth in the epithelial cells in the late stage of infection.

DISCUSSION

Pathogenesis of measles and its associated complications such as giant cell pneumonia, measles encephalitis, and subacute sclerosing panencephalitis are generally considered to be related to immunological phenomena (1, 7, 9). However, only retrospective studies on measles patients have been conducted because of lack of a suitable experimental animal. General similarities in the virological and biological properties of rinderpest virus and those of measles virus led to the present study, in which the possibility of using rinderpest virus infection in rabbits as an experimental model for measles virus infection was investigated.

Histopathological examinations revealed the characteristic lesions consisting of giant cell formation and necrosis of lymphoid follicles in almost all of the lymphoid tissues of the virus-infected rabbits. Among the lymphoid tissues examined, the most marked lesions were consistently observed in the mesenteric lymph nodes and GALT. It is also important to note marked histological changes in the thymus, since histological lesions were rarely detected in measles virus infection in monkeys (15).

The morphology of giant cells resembled those of the Warthin-Finkeldey type observed in measles virus infection in humans and monkeys (16). Necrosis of the lymphoid follicles observed in the rabbits was similar to that reported in rinderpest virus-infected cattle (14). Such lymphoid necrosis is also observed in canine distemper virus infection (13). However, evidence of lymphoid necrosis has not been

reported thus far in measles virus infection in either humans or monkeys (11, 16). As to the genesis of lymphoid necrosis, several mechanisms should be considered: direct viral cytopathic effect caused by virus growth in situ; secondary effect from virus-induced damage of reticular cells which serve as a supporting element of the lymphoid cells; and involvement of corticosteroid released during the infection as suggested in cases of measles and canine distemper viruses (6, 8). Such lymphoid lesions may participate in inducing lymphopenia during infection.

Distribution of virus-specific fluorescence was well correlated with that of the above-mentioned histological lesions. Virus antigen was detected in all lymphoid tissues and in a particularly large amount in the mesenteric lymph nodes and GALT. In accordance with the results of histological examination, the major growth site of the virus is assumed to be these lymphoid tissues situated in close association with the intestinal tissues. Although definite identification of the cell type which supports growth of the virus was not attempted in the present study, immunofluorescent findings suggested that the reticular cells serve as a main target cell. However, the possibility of virus growth in the lymphoid cells cannot be completely eliminated since the present study disclosed the presence of virus antigen near the central arterioles of the spleen, which is considered a thymus-dependent area. Electron microscope examinations of the cattle infected with a virulent rinderpest virus revealed localization of viral nucleocapsids in the reticular cells in large amounts and in the lymphoid cells in relatively small amounts, suggesting an involvement of both cell types as a target of virus growth. The bone marrow, which serves as a source of stem cells, was consistently devoid of virus-specific fluorescence, in agreement with the failure to detect histological changes in the bone marrow. Thus, rinderpest virus may not grow in the stem cells as suggested in the case of measles virus (15).

Fever and diarrhea were the main clinical symptoms consistently observed in rinderpest virus-infected rabbits. Although the degree of fever in children after inoculation of attenuated measles vaccine has been considered as an important parameter in estimating an attenuation level of the vaccine, the mechanism of such virus-induced fever is almost unknown. Since rabbits are the most suitable laboratory animal for an assay of pyrogen, it is anticipated that the present system could be used as an experimen-

tal model for the study of the mechanism of virus-induced fever. Diarrhea as observed in our experiments is not common in measles virus infection except in inconsistent, mild cases (8). A severe necrotic effect of rinderpest virus on the GALT may be speculated to be a primary cause of diarrhea in rabbits. In this sense, the relatively mild effect of measles virus on the GALT as demonstrated by inconsistent formation of giant cells in the GALT (15) might be related to a lack of distinct gastrointestinal disorders in measles virus infection.

Both histological and immunofluorescent examinations indicated a relatively rapid repair process of the virus-induced lesions in the lymphoid tissues. The mechanism of this repair process seems to be very interesting in relation to the possible involvement of host immune responses in recovery from the virus infection. Early production of IF, later replaced by neutralizing antibody, might suggest the importance of these humoral factors in the repair process. However, the possibility of participation of cell-mediated immunity, which was suggested to play a major role in recovery from measles (2) and other virus infections, should also be considered. The relative importance of the two immune systems, humoral and cell mediated, in the recovery from rinderpest virus infection is now under study.

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