牛疫ウイルスの病原性と抗核抗体の産生

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著者	今岡, 浩一 金井, 芳之 西川, 禎一
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Induction of Antinuclear Antibodies in Rabbits by Infection with Rinderpest Viruses with Different Degree of Virulence

Koichi IMAOKA, Yoshiyuki KANAI[1)], Yoshikazu NISHIKAWA, Yasuhiro YOSHIKAWA, and Kazuya YAMANOUCHI

Laboratory Animal Research Center and [1)]Department of Molecular Oncology, Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108, Japan

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Rinderpest virus (RV), belonging to the morbillivirus subgroup of paramyxoviridae, causes acute infections in cattle resulting in high morbidity and mortality. Infection of rabbits with the rabbit-adapted Nakamura III strain of RV (L strain) [5] has been extensively studied for its immunopathological characteristics. In this rabbit model, lymphoid necrosis, severe immunosuppression, and transient production of autoantibodies such as antinuclear antibodies (ANA) and cold hemagglutinin are induced [1, 2, 4, 10, 11]. Recently, viruses with different levels of virulence in rabbits were obtained by *in vitro* adaptation to Vero cells [3]. Accordingly, in this study, certain virological characteristics of these viruses were compared with the production of ANA.

The L strain of RV was prepared as a 10% homogenate of the mesenteric lymph nodes from virus-infected rabbits [10]. The Vero cell-adapted L strain was isolated as described previously [3]. The rabbit-passaged L strain (L_0) had a titer of $10^{3.0}$ median infectious dose (ID_{50})/m*l* when titrated in rabbits [10]. The Vero cell-adapted L strain at the 4th, 8th, 11th, and 30th passage levels (L_4, L_8, L_{11}, and L_{30}) had infectivity titers of $10^{1.8}$, $10^{4.5}$, $10^{6.0}$, and $10^{6.2}$ median tissue culture infectious doses ($TCID_{50}$)/m*l*, respectively [3]. One m*l* of the virus was intravenously inoculated to male New Zealand white rabbits with an average body weight of 2.5 kg.

In the rabbits infected with RV, clinical signs such as fever, diarrhea, and anorexia were frequently observed. Transient fever higher than 40°C was observed 1–4 days post inoculation (p.i.) with L_0, L_4, L_8, and L_{11}. Diarrhea was also observed 3–5 days p.i. with L_0 and L_4 (Table 1).

For histopathological and virological studies, rabbits were killed by exsanguination 5 days p.i.. Lymphoid organs including the thymus, spleen, tonsile, mesenteric lymph nodes and Peyer's patches were fixed in 10% neutral buffered formalin, embedded in paraffin, cut into 4 μm-thick sections, and stained with hematoxylin and eosin (H & E). In the lymphoid organs, lesions were observed in the thymus and peripheral lymphoid tissues including the Peyer's patches, mesenteric lymph nodes and tonsils (Table 1). In infection with the L_0, thymic cortical lymphocytes were almost completely disappeared, and mild necrotic lesions with nuclear debris were also observed in the medulla (Fig. 1A). Among the peripheral lymphoid organs, the Peyer's patches were the most severely affected showing depletion of lymphocytes mainly in the thymus-dependent areas, and severe follicular necrosis with nuclear debris (Fig. 1C). In infection with the L_8, similar lymphoid necrosis and depletion of lymphocytes were observed, but to only a moderate degree (Fig. 1E). In infection with virus of higher passage levels, the severity of the lesions became milder (Fig. 1G). Thus, the clinical and histopathological changes correlated well with the number of virus passages in Vero cells (Table 1).

For the detection of virus antigens by an indirect immunofluorescence (IF) test, small pieces of tissues were frozen in *n*-hexane immersed in a dry-ice acetone bath. Cryostat sections of 5 μm-thick were fixed with acetone at −20°C for 10 min. Serum from a patient with subacute sclerosing panencephalitis (SSPE), which strongly cross-reacts with RV antigens [8], was used as primary antibody instead of anti-RV antibody. Fluorescein isothiocyanate-conjugated goat anti-human IgG (Cappel) was used as the second antibody. In infection with the L_0, a large amount of viral antigens were mainly detected in the medulla of the thymus (Fig. 1B), and in the lymphatic nodules of both the mesenteric lymph nodes and Peyer's patches (Fig. 1D). In the spleen, however, only a few foci of viral antigen were detected in the white pulp. In infection with

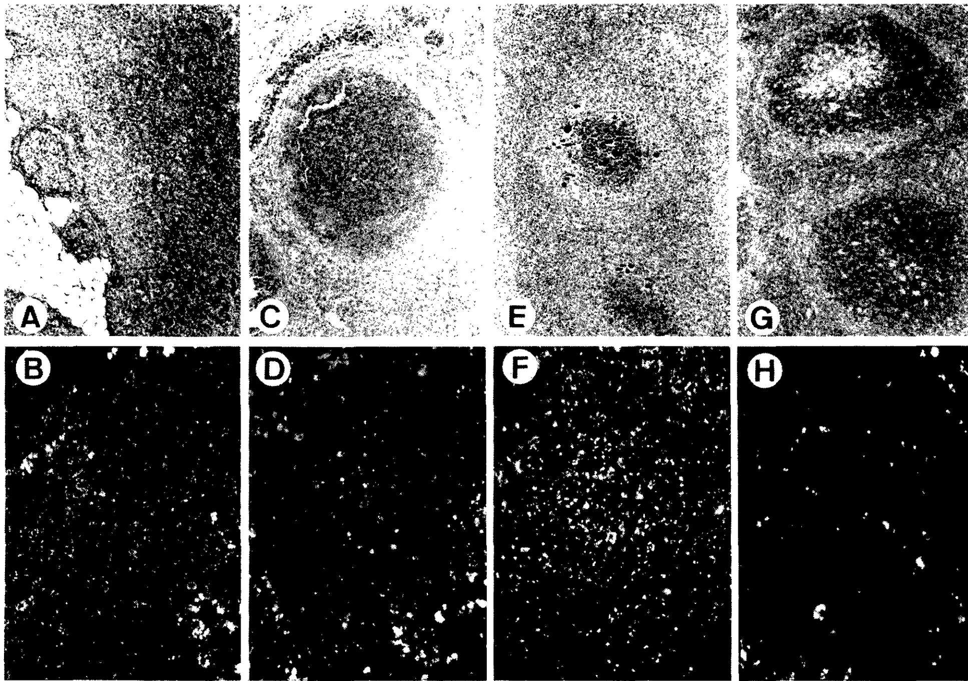

Fig. 1. Histopathological lesions and virus distributions in the thymus (A, B) and the Peyer's patches (C-H) in rabbits infected with the L_0 (A-D), the L_8 (E, F), and the L_{30} (G, H) strains. A: Marked depletion of lymphoid cells from the cortex and nuclear debris in the medulla. B: A large amount of virus antigens in the medulla. C: Severe necrosis and depletion of lymphoid cells. D: A large amount of virus antigens in the lymphatic nodules. E: Moderate necrosis. F: virus antigens around the necrotic lesions. G: Slight depletion of lymphoid cells. H: A few foci of virus antigens. A, C, E, G: HE staining, ×25. B, D, F, H: IF test, ×25.

Table 1. Clinicopathological findings, virus distribution, and incidence of ANAs

Virus	Clinical findings		Lymphoid necrosis			Virus distribution			Indicence of ANAs[d]
	Diarrhea (3–5 dpi[c])	Fever (1–4 dpi)	Spleen	Thymus	Peripheral Lymph nodes	Spleen	Thymus	Peripheral lymph nodes	
L_0	+[a]	+	−	++	+++	+	++	+++	10/10
L_4	+	+	−	+	++	nt[b]	nt	nt	5/5
L_8	±	+	−	+	+	±	+	++	5/5
L_{11}	−	+	−	−	+	nt	nt	nt	4/5
L_{30}	−	±	−	−	±	±	+	+	3/5

a) Clinicopathological changes and virus distribution were graded as +++, ++, +, ±, or − depending on severity of the former, and intensity of virus specific fluorescence for the latter.
b) nt means not tested.
c) dpi means days pi.
d) Indicence of ANAs was judged at 2 weeks pi.

the L_8, viral antigens were detected at the same loci as were found in case of the L_0, but their intensity and dissemination were weaker and more limitted (Fig. 1F). In infection with the L_{30}, a few foci of virus antigen were detected in the thymus, Peyer's patches, and mesneteric lymph nodes (Fig. 1H), but hardly at all in the spleen. These virus distribution pattern seemed to corre-

late well with the histopathological lesions (Table 1).

For the examination of ANA induction, sera were collected from rabbits once a week from 1 to 9 weeks p.i., and inactivated by heating at 56°C for 30 min. The sera were diluted to 1:20 with phosphate buffered saline (PBS, pH 7.4) containing 0.05% sodium azide. As target cells for ANA by the IF test, HeLa cells were grown on coverslips in Leighton tubes, washed with PBS, fixed with acetone at −20°C for 10 min, and stored at −20°C until use. Fluorescein isothiocyanate-conjugated goat anti-rabbit IgG (Cappel) was used as secondary antibody. ANA was detected as a homogeneous nuclear fluorescence. Induction of ANA was observed 1 to 2 weeks p.i. in almost all the rabbits infected with the virus regardless of *in vitro* passage level (Fig. 2). In infection with either the L_0 or L_4, similar ANA responses were observed; titers reaching a maximum 2 weeks p.i. and then disappearing 6–8 weeks p.i.. The ANA response decreased in infection with viruses of higher *in vitro* passage levels. Especially in infection with the L_{11} and L_{30}, a few rabbits produced ANA only for one week or two.

The clinical signs and histopathological lesions by rinderpest viruses with different degrees of virulence were previously reported to change in parallel to the virus adaptation to *in vitro* growth in cultured cells [3]. The present study confirmed this conclusion. Moreover, viral antigens were found to be widely disseminated in the lymphoid tissues of rabbits infected with viruses of the lower passage, but more limited in those infected with virus of higher passage. These results clearly suggest that the damage of lymphoid tissue was directly induced by viral growth, and that the adaptation of RV to Vero cells caused virus attenuation resulting in reduced tropism and viral growth rate in the lymphoid tissues.

Histopathological lesions and viral growth in the spleen were milder than those seen in the thymus, and depletion of lymphocytes was mainly observed in the thymus-dependent area of the peripheral lymphoid tissues. In the thymus, cortical lesions were severer than those in the medulla, suggesting that both mature and immature T cells served as major target cells of RV.

Induction of ANA was observed in almost all the rabbits infected with the virus, though level of induction decreased with increasing virus passage number, and was closely correlated with both severity of the histopathological changes in the lymphoid organs and the virus growth rate. These observations suggest that the production of ANA was associated with the tropism of virus to lymphoid tissues.

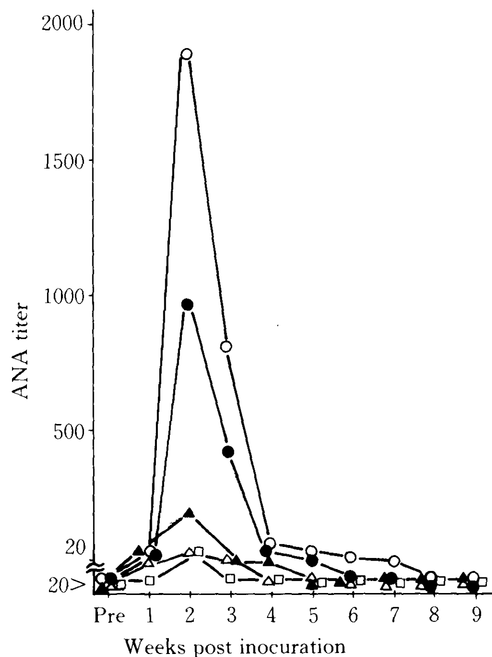

Fig. 2. Mean titers of ANAs measured by IF test in infection with the L_0 (-○-), the L_4 (-●-), the L_8 (-△-), the L_{11} (-▲-) and the L_{30} (-□-) strains.

It has been reported that the production of autoantibodies as well as the development of autoimmune diseases, is associated with certain virus infections [6]. As to the mechanisms of such autoimmune reactions, the following hypothesis have been proposed: 1) release of sequestered antigens, 2) virus-induced alterations of host cell antigens, 3) immunological cross-reactivity between virus components and host antigens, 4) anti-idiotype antibodies to anti-viral antibodies, and 5) disturbance of the immune system [6, 7, 9].

In this study, extensive necrosis of lymphoid tissues was observed, and moreover, nuclear debris were noticed in the lymphoid tissues. Thus, both disturbances of the immune system and the release of large amounts of sequestered antigens may have played important roles in the production of ANA. Since RV is an enveloped

virus showing fusion activity, the possibility of viral modification of host cell antigens as a mechanism for ANA production also remains to be considered.

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要　約

牛疫ウイルスの病原性と抗核抗体の産生（短報）：今岡浩一・金井芳之[1]・西川禎一・吉川泰弘・山内一也（東京大学医科学研究所・実験動物研究施設，[1]癌生物研究部）――牛疫ウイルスウサギ順化株と，Vero 細胞に順化・継代した株をウサギにそれぞれ接種し，リンパ系組織における組織病変とウイルス増殖および，抗核抗体の産生との関係を調べた．組織病変およびウイルス増殖の程度，産生される抗核抗体の抗体価はいずれも，Vero 細胞での継代数が増すにつれて低下した．抗核抗体の産生とウイルスの病原性との相関が示された．