Pathogenicity of a Reovirus Type 2 in Suckling Mice: Insulitis and Lymphoid Atrophy

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Reoviruses produce a variety of histological lesions in suckling mice, including encephalitis, hepatitis, myocarditis, adrenalitis and pancreatitis [11]. Under some circumstances type 1 or type 3 reovirus infects beta cells in certain inbred strains of mice [10, 11]. Acute and chronic diseases of mice with prominent lesions in the liver, pancreas and lungs can be produced in mice infected with Jones strain of reovirus type 2 [13, 14]. Infection of the islet of Langerhans with reovirus type 2, however, has not been observed. Jones strain can produce encephalitis in suckling mice [14]. Histological studies are very limited, but peak injury occurs around day 10 and is maximal in the cortex and thalamic nuclei. Changes have also been observed in the cerebellum and cerebellar peduncles. These available histopathological data in liver, lungs, pancreas and brain of Jones strain of reovirus type 2 infected mice are too limited to permit detailed comparison of the type of disease produced by types 1, 2 and 3 viruses.

In this study, we decided to see pathogenicity of the BN-77 strain of reovirus type 2. The present experiments show BN-77 strain of reovirus type 2 infection in mice lead to beta cell damage. Since reovirus type 1 is also known to induce immunosuppression, histological lesions of thymus and spleen were also studied in BN-77 virus infected mice.

BN-77 strain of reovirus type 2 isolated from a cow with diarrhea [4, 5] was passaged 10 times on the primary bovine kidney cell culture [6]. Then virus was grown on HmLu-1 cell line [6]. The culture fluid was centrifuged at 3,000rpm for 10 min and resulting supernatant was used as virus material.

NC mice were bred at this institute. Two- or 3-day-old 40 mice were inoculated intraperitoneally with 1×10^5 plaque forming units (pfu) of viruses. Half of the infected mice were tested for histopathology and another half were tested for virus isolation. All experiments were carried out

in the barrier-sustained room conditioned at 25°C with humidity of 55%. Mice were fed regular pelleted diet and water *ad libitum*.

Tissues from infected mice were tested for infective virus titers using HmLu-1 cells. Tissue homogenates (1:10) in Eagle's media were prepared from the pancreas, spleen and thymus. And 0.3 ml of ten-fold dilutions for virus titers was inoculated onto the cell cultures in duplicates. The plates were incubated at 37°C for 2 hr and were rocked every 30 min for uniform adsorption. Then 8 ml Eagle's growth medium in 1% agar warmed to 44°C was added to each dish. In this case the growth medium consisted of 10% of FCS. Ten to 15 min later dishes were incubated at 37°C for 6 days. And 5 ml of formalin (1:10) containing 0.05% of methylene blue was added to each plate. After staining at room temperature overnight plaques were counted.

Tissues were fixed in buffered formalin, and 4 μ m-thick paraffin sections were made and stained with hematoxylin-eosin.

No evidence of virus growth was observed in the thymus and spleen of inoculated mice. From the pancreas, however, 10^2 to 10^3 and 10^4 to 10^6 pfu of virus were recovered on days 3 and 5, respectively. On day 7 virus titers were 10^2 and 10^3 pfu, and no virus was isolated on day 14 (Fig. 1).

No distinct histological alteration was observed in the spleen, thymus, lungs, heart and pancreas of infected mice on day 3, 5, 7 of infection. Slightly degenerated hepatocytes were seen in the liver on day 5 and 7 of infection, while no degenerative change was observed in the liver on day 3 of infection.

On day 14 of infection the liver of some cases showed small foci of necrosis with infiltration of neutrophils and lymphocytes. In the pancreas, while, no alteration was observed in acinar cells. There was degeneration in most of islets. Infiltration of neutrophils and mononuclear cells was observed in 80% of the islets (Fig. 2). Some islets had only a few unaffected cells and degenerative changes were observed in less affected ones. In

Fig. 1. Viral growth in the pancreas (●), thymus (○) and spleen (▲) of suckling NC mice infected intraperitoneally with 1×10^5 pfu of virus. Each point represents the mean virus titer from 5 mice; titration was performed on HmLu-1 cells.

Fig. 2. Destruction of pancreatic islets with infiltration of neutrophils and mononuclear round cells, day 14 of infection. $\times 200$.

the myocardium of the left ventricle of one case, epicarditis was observed with infiltration of neutrophils, monocytes and lymphocytes. The lungs were congested with aggregation of lymphoid and reticuloendothelial cells, in the interstitium and around vessels and bronchioles. Occasionally alveolar hemorrhage was observed.

The thymus, spleen and lymph nodes were severely atrophied. The thymus in all cases examined showed thymocyte depletion in the cortical area, which became almost empty in severely affected cases (Fig. 3). The medullary epithelial elements with cellular infiltration were more prominent as compared to that of age matched controls. Slight fibrosis was observed. In the spleen, lymphoid depletion was seen in the thymus-dependent area as well as medullary cord without degeneration or necrosis (Fig. 4). No significant lesion was observed in the brain, kidneys and salivary glands.

Infection with Jones strain of reovirus type 2 in mice has been studied by some authors [13, 14]. With Jones strain of virus myocardial and pulmonary lesions were described to be more prominent, while the nervous system was less

Fig. 3. Depletion of thymocytes in the thymus cortex and prominent epithelial elements in the medulla, day 14 of infection. $\times 200$.

Fig. 4. Depletion of lymphocytes and increased number of giant cells in the spleen, day 14 of infection. $\times 200$.

affected than in the case of reovirus type 1 or type 3 infections. In this study using BN-77 strain of reovirus type 2, lymphoid atrophy with progressive cortical lymphocyte depletion and destruction of pancreatic islets were prominent, whereas myocardial and pulmonary lesions were occasional, and so far no histological lesion was observed in the nervous system. Such lymphoid involvement has not been previously described in reovirus infections except for a very brief note in reovirus type 1 infection [11].

Lymphoid atrophy has been described in many virus infections in mice [1-3, 8, 9, 12]. In these studies direct effect of viral growth in the lymphoid system was an important factor in lymphoid atrophy. In this study no reovirus growth in the thymus and spleen was evidenced. However in coxsackievirus B3 (CB3) infected mice lymphoid atrophy progressed after clearance of the virus from the organs, and autoimmunity was suggested for pathogenesis of lymphoid atrophy and myocarditis [7]. Similar findings were also reported in reovirus type 1 infected mice [10]. Same mechanisms as in CB3

and reovirus type 1 infection may be responsible for the lymphoid atrophy in BN-77 strain of reovirus type 2 infection.

Pancreatic insulinitis was previously reported in reovirus type 1 or type 3 infected mice [10, 11]. However, Jones strain of reovirus type 2 was described to induce minor changes only in acinar cells [14]. For isolation of diabetogenic reovirus type 1 or type 3, original virus pool has been made by passage in pancreatic beta cell cultures [10, 11]. In this study BN-77 strain of reovirus type 2, which had no history of passages in pancreatic beta cells in mice, was revealed to induce insulinitis without involvement of acinar cells, suggesting that BN-77 strain of reovirus type 2 might be more diabetogenic than reovirus type 1 or type 3. Ongoing experiments showed that BN-77 strain induced an abnormal glucose tolerance test in several strains of inbred mice (unpublished observations).

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要 約

レオウイルス 2 型の乳のみマウスにおける病原性：脾臓炎およびリンパ組織萎縮 (短報)：小野寺 節・谷口稔明・吉原一浩・佐藤真澄・本間惣太 (家畜衛生試験場)——2 型レオウイルス BN-77 株を接種された 2-3 日齢 NC マウスでは、胸腺および脾臓におけるウイルスの増殖は観察されなかったが、脾臓においてはウイルス増殖がみられ、 10^5 pfu/脾に達した。脾臓には脾臓炎がみられたが、外分泌細胞の変化は観察されなかった。軽度の肝炎、肺炎および心筋炎と、全身リンパ組織における重度の萎縮が観察された。