Detection of Nucleoprotein Gene of Sendai Virus in the Lungs of Rats by Touchdown Nested Reverse Transcription Polymerase Chain Reaction

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Abstract: The nucleoprotein (NP) gene of Sendai virus was detected by touchdown nested reverse transcription polymerase chain reaction (RT-PCR) in the lungs of a rat presented with respiratory illness and high serum ELISA titer to Sendai virus. This method seemed to be of value in controlling infection in laboratory rodents.

Key words: hemagglutinating virus of Japan, laboratory rodents, paramyxovirus

Sendai virus (HVJ: hemagglutinating virus of Japan) is a negative sense RNA virus which belongs to the family *Paramyxoviridae*. Although Sendai virus infection used to be enzootic among laboratory rodents [6, 7, 10], nowadays Sendai virus-free rodents are provided by breeders. In our facility, the quality of these laboratory rodents is monitored by ELISA titers to Sendai virus [4, 8, 11] and other pathogens. When high titers of antibodies to Sendai virus are noted, it is necessary to distinguish new infection from infection in the past. Evidences of current Sendai virus infection can be obtained by isolation of viruses through embryonated chicken eggs or cultured cells such as LLC-MK₂ cells, detection of viral antigens or an increase in antibody titers. Although detection of Sendai virus genes by PCR is not utilized commonly in controlling laboratory rodents due to its expensiveness, it is important to establish a rapid diagnostic system for Sendai virus

infection because this virus is so contagious that an entire colony could be contaminated rapidly. Eradication of the Sendai virus from an infected mouse colony is successfully done by sacrificing the breeder mice and offsprings [3, 9], Caesarean section [12], and by isolating the mouse colony from weanlings and pregnant mice with antibodies [5].

Recently the rodents in our Laboratory of Experimental Medicine were reported to have a high ELISA titer to the Sendai virus, and some of them presented with ruffled fur, and emaciation. This episode prompted us to establish rapid diagnostic methods for Sendai virus. From the lungs of a rat with respiratory illness, we recently tried to amplify a nucleotide sequence of Sendai virus by nested RT-PCR, applying a touchdown method [2] in the first PCR. The breeder of the rodents used in this study was Japan Slc, Hamamatsu. A 6-week-old male SD rat (R5) which presented with respiratory ill-

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ness and high serum ELISA titers to Sendai virus, an asymptomatic 6 week-old female Wistar rat without a high serum ELISA titer to Sendai virus, and an asymptomatic 8 week-old male Donryu rat without a high serum ELISA titer to Sendai virus were sacrificed. Extraction of RNA from the homogenized lung was performed based on a method described elsewhere [1]. Ethanol-precipitated RNA was dissolved in 100 μ l of DDW. Twenty microliters of this RNA suspension was mixed with 42 μ l of DDW and 15 μ l of 150 mM Tris HCl pH8.3 before denaturing at 90°C for 3 min. Eight microliters of 25 mM dNTP, 8 μ l of 10X Mg, K solution (100 mM MgCl₂, 1 M KCl), 2 μ l of 80 mM dithiothreitol, 1 μ l of sense primer (HVJ1S), and 2 μ l

of reverse transcriptase derived from Molony murine leukemia virus (Wako, Tokyo, Japan) were added and incubated at 42°C for one hour. Synthesized DNA was extracted with phenol-chlorform, ethanol-precipitated, and dissolved with 50 μ l of DDW. The DNA sequences of the primer HVJ1S, HVJ1AS, HVJ2S and HVJ2AS were based on the nucleotide sequence 92–107, 776–761, 138–157, and 386–367 of Sendai virus, respectively (Fig.1). HVJ1S, and HVJ1AS were used as the outer set. HVJ2S and HVJ2AS were used as the inner set. The first PCR was performed by a touch-down method with decreasing annealing temperature every three cycle on a DNA Thermal Cycler (Perkin-Elmer-Cetus, Norwalk, CO., U.S.A.). A mixture of 76

Fig. 1. Primers for RT-PCR of Sendai virus.

Fig. 2. Touchdown nested RT-PCR of Sendai virus NP gene from rat lungs. Lane 1, molecular weight marker; lanes 2, 5, and 7, the first PCR with primer HVJ1S and HVJ1AS; lane 3, the first PCR with primer HVJ2S and HVJ2AS; lanes 4, 6 and 8, the second PCR. Lanes 2, 3 and 4, lungs of a rat presented with respiratory illness and high ELISA titer to Sendai virus; lanes 5 and 6, lungs of an asymptomatic Wistar rat; lanes 7 and 8, lungs of an asymptomatic Donryu rat.

Fig. 3. Sensitivity of the touchdown nested RT-PCR of Sendai virus. RNA was extracted from the serially diluted Sendai virus suspension and the touchdown nested RT-PCR was performed as described in the text. Lane 1, molecular weight marker; lane 2, 0.01 HAU/50 μ l; lane 3, 0.001 HAU/50 μ l; lane 4, 0.0001 HAU/50 μ l; lane 5, 0.00001 HAU/50 μ l.

μ l of DDW, 10 μ l of 10X PCR buffer, 10 μ l of 2.5 mM dNTP, and 1 μ l of Taq polymerase (Takara, Kyoto, Japan), 1 μ l of outer-set primers or inner-set primers was overlayed with one drop of mineral oil. After heating at 95°C for 30 sec, three cycles of denaturing at 94°C for 1 min, annealing at 44°C for 30 sec, and elongation at 72°C for 2 min were performed. The annealing temperature was decreased by 2°C every three cycle and finally, 15 cycles with an annealing temperature of 36°C were performed. By performing the first PCR by the touchdown method, which allows less contingent annealing of primers to templates, the sensitivity of the nested PCR is thought to be improved. The second PCR was performed with 1 μ l of the inner set primers and 1 μ l of the first PCR products. After heating at 95°C for 30 min, 25 cycles of denaturing at 94°C for 1 min, annealing at 44°C for 30 sec, and elongation at 72°C for 2 min were performed.

Although in the first PCR, neither a 684 nor a 248 base pair band was detected, the second PCR clearly visualized a 248 base pair band from the lungs of SD rat R5, indicating the existence of the Sendai virus NP gene. From the lungs of two other rats, no Sendai virus-specific band was detected (Fig. 2). The sensitiv-

ity of the touchdown nested PCR was determined with the Fushimi strain of the Sendai virus. The Sendai virus NP gene could be detected from 0.0001 HAU/50 μ l of virus suspension by the touchdown nested RT-PCR (Fig. 3). This method seems to be of value in controlling the infection of rodent breeding colonies with the Sendai virus because the current infection can be distinguished from the infection in the past by detecting the viral RNA within 24 hr.

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