Short Communication

**IMMUNOFLUORESCENCE FOR DETECTION OF VIRAL ANTIGEN
IN MICE INFECTED WITH SENDAI VIRUS**

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SUMMARY: Sendai virus infection transmitted by contact from cagemates was followed by virus titration and immunofluorescence. The virus grew in the respiratory tract and caused macroscopic lesions in all contact mice. The virus grew to a higher titer in the lung than in the trachea. Tracheal smears, however, were found to be the most suitable for the diagnosis of Sendai virus infection by immunofluorescence, since they contained a large number of cells with intense fluorescence. Diagnosis of Sendai virus infection was made by immunofluorescence within a few hours after autopsy made at early stages of infection.

Because Sendai virus infection is widespread among laboratory mice and rats in Japan (Fukumi, Mizutani and Takeuchi, 1962), its prevention is mandatory to maintain animals in clean states. Rapid diagnosis, removal of infected animals and disinfection are important measures for the purpose. Detection of viral antigens by direct immunofluorescence (Coons et al., 1942) has been utilized for diagnosis of infection of animals with influenza virus (Liu, 1955), rabies virus (Goldwasser and Kissling, 1958) and many other viruses (Emmons and Riggs, 1977). Although immunofluorescence has been employed by a number of investigators (Blanford and Hearsh, 1972; Shimokata et al., 1976) to study the pathogenesis of Sendai virus infection, its application to the diagnosis has not been reported. We followed the course of Sendai virus infection in mice, which closely simulated natural spread of the virus among laboratory animals, and found the immunofluorescence to be particularly suited for detecting the infection at early stages.

Sendai virus, M-73 strain, isolated by us from a naturally infected mouse in a breeder colony (Nakagawa et al., 1980) was used. The virus was propagated either in 10-day-old embryonated chicken eggs or primary cultures of cynomolgus monkey kidney (MK) cells. To prepare the material for immunization, Sendai

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virus concentrated from infected allantoic fluid by pelleting was purified by centrifugation at 35,000 rpm for 2 hr through 15 to 60% sucrose gradient (Duesberg and Robinson, 1965). Rabbits received five intravenous injections of purified virus at 2-day intervals for priming. Four weeks after the 5th injection, a booster injection was given intravenously with partially purified virus grown in MK cells. Rabbits primed and boosted with egg-grown virus produced a high level of antibody against normal chick cell components. By following the immunization schedule described above, we obtained the sera with sufficiently high antibody titer against Sendai virus but with negligible antibody titer against normal chick cell components. Antisera were labeled with fluorescein isothiocyanate according to Kawamura (1969) and Shimojo, Yamamoto, and Abe (1967). The staining titer of the fluorescein-conjugate determined in MK cells infected with Sendai virus ranged from 1:8 to 1:40. Specific pathogen-free, 4-week-old female mice of the ICR strain were used. Five healthy mice (contact) were housed in one cage together with a mouse (infectior) infected by nasal instillation of 5×10^6 TCID₅₀ of Sendai virus 3 days earlier. Cages were kept in a vinyl isolator throughout the experimental period of 18 days to prevent infection with other pathogens. Mice were given irradiated pellet food and tap water *ad libitum*. Each day after the beginning of contact, three contact mice were sacrificed and examined for gross lung lesions, the presence of viral antigen in the respiratory tract, virus growth and antibody response. Viral antigen was tested by immunofluorescence of nasal, tracheal and lung smears. Virus

Fig. 1. Course of Sendai virus infection in mice. Virus and antibody titers are geometric means of those of three mice.

Lung lesion: Number of mice with macroscopic lung lesions/Number of mice examined.
 ●—●: virus titer in the trachea, ○---○: virus titer in the lung, △---△: antibody titer.

TABLE I

Detection of viral antigen by immunofluorescence in the respiratory tract of mice infected with Sendai virus

Tissue		Specific fluorescence Days after the beginning of contact														
		1	2	3	4	5	6	7	8	9	10	12	14	16	18	
Nose	Intensity	+	±	+	+	+	+	±	±	—	±	±	—	—	—	
	%	25	10	25	25	25	25	10	10	0	10	10	0	0	0	
Trachea	Intensity	—	+	++	++	++	++	+	+	—	—	—	—	—	—	
	%	0	25	50	50	50	50	25	25	0	0	0	0	0	0	
Lung	Intensity	—	—	+	+	++	+	+	—	—	—	—	—	—	—	
	%	0	0	25	25	50	10	25	0	0	0	0	0	0	0	

—: No fluorescence; ±: Faint fluorescence; +: Distinct fluorescence.

++: Intense fluorescence.

%: Shows the percentage of the fluorescent cells in a smear.

Three mice were sacrificed on each day.

contents of the trachea and the lungs of each mouse were determined by infectivity titration. The trachea and the lung were each made into a 10% suspension and 0.2 ml of each of serial 10-fold dilutions was inoculated into tube cultures of MK cells. After incubation at 36 C for 10 days, cultures were tested for hemadsorption with guinea-pig erythrocytes. Infectivity was calculated by the method of Reed and Muench (1938) and expressed in $\text{TCID}_{50}/0.2 \text{ ml}$. Antibody titration was made with individual mice by the complement fixation test using Sendai-infected allantoic fluid as antigen. The test was performed by the Kolmer's method as reported by Shimojo et al. (1966).

Contact mice showed overt signs of rhinitis but infection was not fatal. The course of infection is shown in Fig. 1. Virus was recovered from the trachea from day 3 to day 7, while from the lung from day 2 to day 9. The peak virus titer was $10^{1.3} \text{ TCID}_{50}/0.2 \text{ ml}$ in the trachea and $10^{4.3} \text{ TCID}_{50}/0.2 \text{ ml}$ in the lung. The lung lesions appeared in one of three mice on day 3 and were seen regularly in all mice from day 7 onward. Antibody developed in all three mice from day 9. Thus, all contact mice became infected with Sendai virus. The proportion of the cells with viral antigen as demonstrated by immunofluorescent staining of smears of the respiratory tract and the intensity of fluorescence are shown in Table I. A small number of cells with faint fluorescence were seen in nasal smears from day 1 and persisted intermittently until day 12. In the trachea, antigen-containing cells appeared on day 2, increased both in number and intensity of fluorescence during the period from day 2 to day 6, and were detectable until day 8. Antigen-positive cells, observed from day 3 to day 7, were fewer in number and less intense in fluorescence in the lung than those in the trachea. A tracheal smear stained with anti-Sendai fluorescein conjugate is shown in Fig. 2. Specific fluorescence of Sendai virus is seen in the cytoplasm but not in the nucleus.

Fig. 2. A smear from the trachea taken on day 3, stained with anti-Sendai conjugate. Irregular fluorescent granules are seen in the cytoplasm but not in the nucleus. $\times 200$.

We conclude from these results that the immunofluorescent staining of tracheal smears provides a convenient method for rapid diagnosis of Sendai virus infection in mice in its early stages before the development of lung lesions and antibody. We have applied the method to the screening of mice for Sendai virus infection and found it useful for the purpose (unpublished data).

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