

Isolation of a Parainfluenza Virus Type 3-like Agent from Guinea Pigs

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Guinea pig (GP) sera seropositive to human parainfluenza virus type 3 (PIV-3) antigen were found previously in our laboratory. In the present study, we attempted to isolate and characterize a new agent from GP colonies. The new agent was isolated from lung homogenate of sentinel GPs, which were maintained in the GP room containing the serologically positive GP breeding colony. This new agent was found to be comparable to human and bovine PIV-3 strains in regard to its moderate cytopathic effects, hemagglutinating activity, and neutralizing activity, and moreover, it was found to produce antigens cross-reactive to both of the latter PIV-3s. The resemblance between the new isolates and the other PIV-3s was supported by the SDS-PAGE and Western blot analysis of the viral proteins. This is the first report of the isolation of a type 3-like parainfluenza virus from GP. This finding is significant, insofar as an inapparent infection with the virus may concern animal experimentation in the use of GP.

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Introduction

Parainfluenza viruses (PIVs) belong to the family *Paramyxoviridae* and are important respiratory tract pathogens in human and non-human animals. The PIVs include at least four different serological types (PIV types 1-4). PIV types 1, 2, and 3 are a major cause of croup and bronchiolitis in infants and children¹⁻³⁾. Animal strains of PIVs have been reported in mice (murine PIV-1; Sendai virus)^{4, 5)}, cattle (bovine PIV-3)⁶⁾, and dogs⁷⁾, but not in guinea pigs (GP). Serological evidence has been reported and which suggested the infection of GP with PIV-3^{8, 9)}. However,

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there have as yet been no published reports of the isolation of PIV-3 from GP. GPs seropositive to both human and bovine PIV-3s antigens were recently found in our laboratory. In the present study, we therefore attempted to isolate and characterize a new PIV-3 strain from these seropositive GP.

Materials and Methods

Sentinel animals

Sentinel animals (4-week-old germ-free GP of the Hartley strain) supplied by Nihon SLC (Hamamatsu, Shizuoka, Japan) were maintained in the breeding room containing GP that were serologically reactive to human and bovine PIV-3 antigens. At 8, 10, 12, and 14 days of exposure, one of the four each animal was placed under general anesthesia with diethyl ether or pentobarbital for bleeding and euthanasia. The blood and lung specimens were collected for serological tests and virus isolation. These procedures were conducted under the Guidelines for the Animal Care and Use Committee of Nagasaki University.

Viruses and culture cells

The 65-899 strain of human PIV-3 (provided by courtesy of Dr. Y. Ito, Mie University School of Medicine), the C243 strain of human PIV-3 (purchased from American Type Culture Collection, Rockville, MD, USA), the 910N and YN strains of bovine PIV-3 (donated by Dr. Y. Iwakura, Institute of Medical Science, University of Tokyo), simian virus type 5 (SV-5), and murine PIV-1 (both provided by the late of Dr. M. Nakagawa and Dr. M. Arita, National Institute of Health, Japan) were used as control PIVs. The growth and maintenance of culture cells and the method of serological tests were in accordance with the previous description¹⁰⁾. In short, the B95a, BHK-21/clone 13, BSC-1, MA-104, MDBK, and Vero cell lines were maintained in Eagle's minimum essential medium (MEM; Gibco Co., Grand Island, NY, USA)

containing 10% or 5% heat-inactivated fetal calf serum (FCS; Hyclone Lab., Logan, UT, USA) and antibiotics. For the production of stock virus and hemagglutinin (HA), the cells were inoculated with each of the six viruses at a multiplicity of infection of about 1. The supernatants were employed in hemagglutination-inhibition (HI) and viral neutralization (VN) tests as virus antigen.

Virus isolation

The removed GP lungs were suspended with MEM with 5% FCS (5MEM) and prepared as a 10% (w/v) final concentration in an ice-bath using a glass homogenizer at 4,000 rpm for 1 min inside a biological safety cabinet (Class II). Aliquots of the homogenates were centrifuged at 2,000 rpm at 4 °C for 10 min, and two-fold dilutions of the supernatants were inoculated into six different subconfluent cells. After the adsorption with supernatants, the inoculated cells were rinsed with 5MEM and cultured in a CO₂ incubator at 37 °C. These cells were observed daily for cytopathic effects (CPE), and examined for an appearance of viral antigen by indirect immunofluorescent antibody (IFA) test.

Characterization of isolates

A plaquing method was employed for measuring the PIVs' infectivity. The new isolates were examined in detail to determine their type of viral nucleic acid, sensitivity to ether treatment, HA production, hemadsorption (HAD) activity, and serological cross-reactions using VN and HI tests. The viral nucleic acid was identified via treatments with an acridine orange 7 and 5-iodo-2'-deoxyuridine (Wako Chemicals Co., Osaka)¹¹. The ether sensitivity and HI tests were performed according to the procedures described previously¹². For the detection of HA activity, a 0.5% erythrocyte suspension was used. The PIV-inoculated cells were also examined with HAD test to detect the HA antigen on the surface membrane of the cells tested.

Sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis (SDS-PAGE) and Western Blotting: The purified antigens were mixed with an equal volume of 2x SDS sample buffer (0.1 M Tris-HCl pH 6.8, 20% glycerol, 4% SDS, 2% 2-mercaptoethanol, and 0.001% bromophenol blue) heated at 100 °C for 2 min, and the aliquots were loaded onto a 10% acrylamide gel and electrophoresed at 120 volts for 4 h. Proteins on the separated gel were either stained with Coomassie blue or transferred to a nitrocellulose membrane (Millipore,

Bedford, MA, USA) at 6 volts overnight using a Semidryblotter (Sartorius, Gottingen, Germany). The protein bands were visualized by anti-new isolates antisera of rabbits and 4-chloro-1-naphthol substrate obtained from a commercial kit (Vector, Burlingame, CA, USA).

Results

Four GPs confirmed to be seropositive to human PIV-3 were selected for the isolation of a new agent. Three-fourths of this sentinel GP group had shown seroconversion (1:16 in HI titer) 14 days after introduction into the contaminated colony. At autopsy, no apparent disorders or consolidations were observed in any of the GP lungs. Lung homogenates of the four GPs were inoculated into B95a, BHK-21/clone 13, BSC-1, MA-104, MDBK, and Vero cells, and the cells were observed daily and examined for the viral antigen using the IFA test. The new isolates induced moderate CPE with cell fusions in B95a cells, and rounding cell formation in MDBK and BHK-21/clone 13 cells on and after 2 days of incubation (Fig. 1). Moreover, IFA antigens were observed in all cells after 1 day post-inoculation (dpi). Three viral isolates were finally obtained from the GP lungs taken on the 8th, 10th, and 12th day of the group housing. The viral nucleic acid of new isolates was demonstrated to be single-

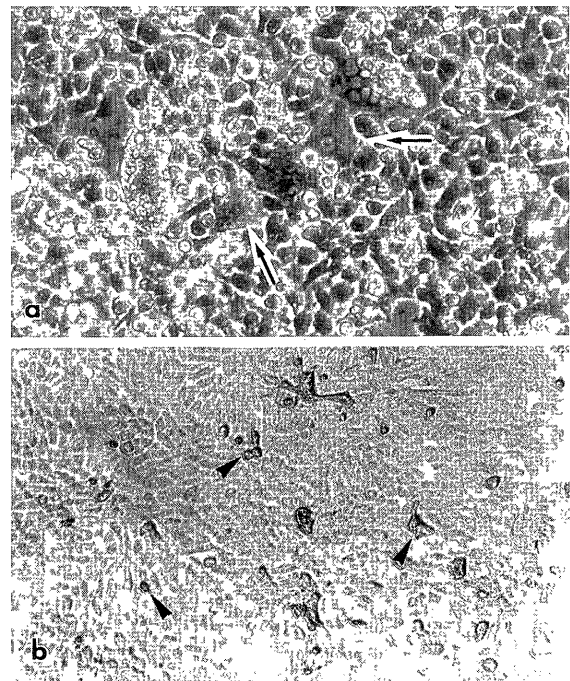


Fig. 1. Cell fusion (arrows) induced by the isolate in B95a cells (a), and rounding cell formations (arrowheads) in MDBK cells (b) at 2 days post-inoculation (dpi). a, b: x 150.

stranded RNA, as determined by acridine orange staining and by treatment with 5-iodo-2'-deoxyuridine. A marked reduction in infectivity was observed after treatment with diethyl ether for 18 h at 4 °C, indicating the ether-sensitive nature of the virus isolates. The virus isolates were also examined for HA production with the erythrocytes of four mammalian species (mice, rats, GPs, and rabbits). Like human strain (65-899) and bovine PIV-3 (strains 910N and YN), the isolates agglutinated all of the erythrocytes. No difference in the HA titers and optimal temperatures for HA production were noticed with the erythrocytes from these animals. In HAD test using B95a and

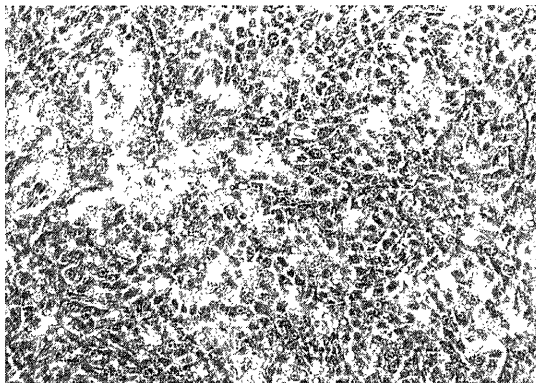


Fig. 2. In the hemadsorption test by MDBK cell on 2 dpi, the fusions surrounded with the many rounded cells adsorbed many GP erythrocytes (small round cells). x 150.

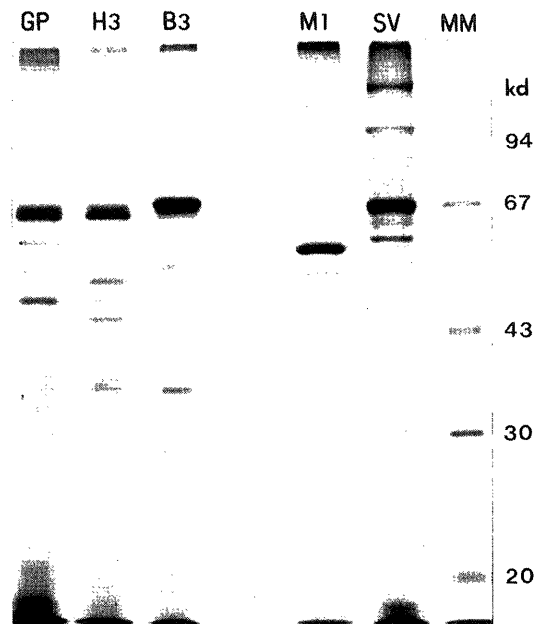


Fig. 3. SDS-PAGE analysis of the isolate, human PIV-3, bovine PIV-3, murine PIV-1, and SV-5. GP: new isolate; H3: human PIV-3; B3: bovine PIV-3; M1: murine PIV-1; SV: simian virus 5; and MM: molecular size marker.

MDBK cells, the fusions surrounded with the many rounded cells adsorbed many GP erythrocytes (Fig. 2).

Serologically, the new isolates were distinguished in HI and VN tests from murine PIV-1 and SV-5, insofar as the hemagglutination inhibition and neutralization activities were observed in only three strains of PIV-3 (data not shown). The resemblance between the isolates and other PIV-3s was supported by the SDS-PAGE analysis of the viral proteins (Fig. 3). In the Western blot analysis, the anti-new isolate rabbit antiserum showed many reactive bands in three strains of PIV-3 (Fig. 4), i.e., putative proteins of phosphoprotein (P), hemagglutinating glycoprotein (HN), fusion protein (F), nucleocapsid protein (NP), and others. Anti-new isolate matrix protein (M) antibody was not reactive with bovine PIV-3.

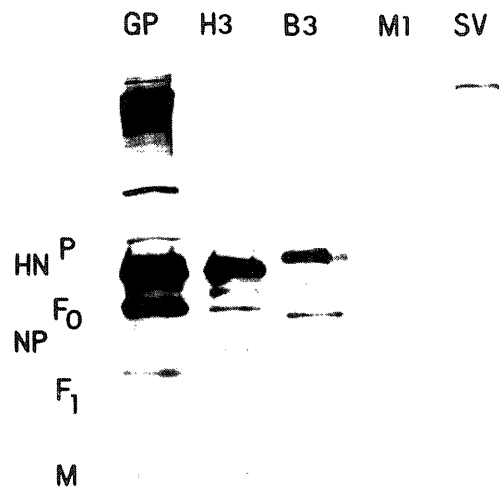


Fig. 4. Western blot analysis of the isolate, human PIV-3, bovine PIV-3, murine PIV-1, and simian virus 5 (SV-5). Anti new isolate rabbit antiserum produced many reactive bands in the GP, human, and bovine PIV-3, but not in the murine PIV-1 or SV-5. Abbreviations at the left side show putative proteins; P: phosphoprotein; HN: hemagglutinating glycoprotein; F0: uncleaved fusion protein; F1: large-subunit polypeptide of the cleaved fusion protein; NP: nucleocapsid protein; and M: matrix protein.

Discussion

No reports of PIV-3 isolated from GP have to date been published, even though serological evidence suggesting the infection of GP with PIV-3 has emerged^{8,9)}. While the Sendai virus and SV-5 are animal strains of PIV-1 and PIV-2, respectively, there is no specific animal strain of PIV-3 in GPs. In this study, sentinels were introduced as roommates (not cagemates) to the

breeding room of GP, resulting in the isolation of a new agent which shared biological and physicochemical characteristics with both human and bovine PIV-3s (especially human PIV-3). To our knowledge, this is the first report of a PIV-3-like agent isolated from a GP colony.

Although the GP sentinels in this study displayed no clinical signs after they were reared in the contaminated breeding room, histological and biochemical findings suggesting inflammatory responses in airway, i.e., interstitial pneumonia¹³⁾, an increased number of inflammatory cells in pulmonary lavage fluid¹⁴⁾, an increase in the histamine release from basophils¹⁵⁾, and airway hyper reactivity to histamine^{14, 16)} have been observed in PIV-3 infected GP. PIVs are usually transmitted by aerosol droplets or by direct or indirect contact with respiratory secretions, like other respiratory viruses.

In this report, it seemed that sentinel GPs were horizontally infected via PIV-3 aerosol droplets. This raises the possibility that an inapparent infection with the new isolates may concern animal experimentation in the use of GP⁹⁾. Further experimental examination is needed to clarify the effect of the virus infection in GP.

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