Viremia During Parainfluenza Type 3 Virus Infection of Hamsters[1]
(37673)

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Isolation of parainfluenza viruses from blood during natural infection in man has been reported infrequently (1–3). In the first demonstration of viremia in human parainfluenza Type 3 virus (Para 3) infections, this virus was isolated from whole blood samples obtained from 3 of 83 children within 24 hr of hospital admission with acute respiratory disease (3). During 1968–69, an outbreak of Para 3 occurred in a group of adult males who were being followed in a study of the pathogenesis of chronic bronchitis and emphysema. Twenty-three of 29 subjects became infected and Para 3 was isolated from clotted blood, or various components of heparinized blood on 26 occasions from 19 individuals (1).

Although only a few studies of viremia in animals experimentally infected with parainfluenza viruses have been conducted (4), an excellent model for asymptomatic infection of hamsters with Para 3, following intranasal inoculation of "unadapted" tissue culture strains of the virus, has been described by Craighead, Cook and Chanock (5). By immunofluorescent staining, viral antigen was localized in the cytoplasm of infected ciliated bronchial epithelial cells (6). However, the occurrence of viremia has not been reported in this model (5–7).

In view of the relative infrequency with which viremia has been found in man during the course of myxovirus infections, viremia in the hamster model of Para 3 infection was studied in order to provide additional information about the pathogenesis of this infection, and as a means of improving methods for isolating myxoviruses from the blood of patients.

Materials and Methods. Virus strain. The virus used was a strain of Para 3 isolated from the throat swab of a patient during an acute exacerbation of chronic bronchitis. It had been passaged three times in WI-38 cells, and five times in rhesus monkey kidney cells. Stock virus, stabilized in 10% DMSO (8), was kept frozen at —60° in small aliquots. Freshly thawed virus had an HA titer of 1:640, and a $TCID_{50}$ titer of 10-4 in rhesus monkey kidney cells, as calculated by the method of Reed and Muench (9).

Animal inoculation and sampling. One month old golden Syrian hamsters of either sex, weighing about 70 g each were housed in individual cages. Animals were lightly anesthetized with ether and then allowed to aspirate intranasally 0.1 ml of tissue culture fluid containing 10^4 $TCID_{50}$ of Para 3. At intervals of from 1 hr to 3 wk after inoculation, animals were anesthetized, the chest cavities were opened, and exsanguination was performed by means of intracardiac puncture (utilizing a heparinized vacutainer tube with a 20 gauge, 1.5 in. needle, Becton-Dickinson and Co., Rutherford, NJ). Blood samples and tissue specimens were obtained from groups of three animals and pooled. Between 2.0 and 3.0 ml of whole blood was mixed with 1.0 ml of 3% gelatin, and allowed to stand for 1.5 to 2 hr at room temperature. The supernatant leukocyte–plasma mixture was separated from the erythrocytes by aspiration with a Pasteur pipette, and the leukocytes were re-

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moved from the plasma by centrifugation at 1000 rpm for 10 min, at room temperature. Leukocytes and erythrocytes were washed and resuspended in cold phosphate buffered saline (PBS). Pneumonectomies were performed aseptically, and the lungs were washed in cold PBS, ground in 5 ml cold PBS and centrifuged for 10 min at 1000 rpm. The supernatants from the suspension were utilized as inoculum for tissue culture. Liver tissue was prepared for tissue culture inoculation in a manner similar to that for lung.

Isolation of virus from inoculated animals. Two-tenth milliliter aliquots of fresh erythrocytes, leukocytes, plasma and finely ground lung and liver tissue were inoculated into duplicate rhesus monkey kidney and hamster kidney tissue culture tubes with human kidney growth medium containing 10% fetal bovine serum (10). Tissue culture tubes inoculated with erythrocytes, lung, or liver tissue were allowed to incubate at 37° for 18 hr; they were then washed with PBS and replenished with fresh medium. Cultures inoculated with plasma were washed with PBS and replenished with medium after 4 hr incubation. Leukocytes were allowed to remain on the tissue culture cell layers without subsequent washing. At the end of 6 days incubation at 37°, both inoculated and uninoculated control tissue cultures were washed with cold PBS, and tested for hemadsorption with a 0.5% suspension of fresh guinea pig erythrocytes (11).

Virus recovered from lung tissue at intervals of 24 to 120 hr was quantitated by inoculating serial 10-fold dilutions into rhesus monkey kidney tissue culture tubes, testing for hemadsorption after 6 days, and determining the 50% endpoint of infectivity/0.1 g tissue (9). Lung tissue used for this purpose had been stored at —60° in 10% DMSO.

Antibody determinations. Hemagglutination-inhibiting (HI) antibody titers were determined in pooled plasma from groups of 3 experimental animals sacrificed at various intervals, up to 3 wk after Para 3 inoculation; neutralizing antibody titers were determined in plasma obtained from animals prior to and 3 wk after inoculation, utilizing 100 $TCID_{50}$ of stock Para 3 virus (11). Plasma neutralization titers were also obtained 3 wk following a second intranasal inoculation with 10^3 $TCID_{50}$ Para 3, given 3 wk following the initial intranasal inoculation.

Results. Observations after infection of animals. Animals remained well during 3 wk of observation following intranasal inoculation of Para 3. Lung, liver, and spleen tissue appeared grossly normal when animals were sacrificed at various time intervals. Standard sections of lung tissue obtained at 24 hr intervals from 24 to 168 hr following inoculation, stained with hematoxylin and eosin, appeared normal, except that some of the bronchioles contained an excess of sloughed epithelial cells. There was no evidence of inflammatory cell exudate in bronchial or alveolar spaces. Sections of lung obtained at 3 wk following inoculation showed focal areas of peribronchiolar interstitial fibrosis.

Recovery of virus from animals. As shown in Table I, at every time interval virus was found in lung tissue obtained from groups of 3 animals sacrificed at 24 through 120 hr, after intranasal inoculation of 10^4 $TCID_{50}$ of Para 3. However, no virus was found in lung tissue from animals sacrificed at 1 and 3

TABLE I. Isolation of Parainfluenza Type 3 Virus from Hamsters Infected by the Intranasal Route.[a]

Material tested for virus	After inoculation (hr)											
	1	3	4	24	48	72	96	120	140	168	216	504
Lung	0	0		+	+	+	+	+	0	0	0	
Liver	0	0		0		0	+		0			
Plasma	0	0	0	0	0	0	0	0	0	0	0	0
Erythrocytes	0	0	+	+	+	0	0	+	0	0	0	0
Leukocytes				0	0			0		0	0	0

[a] +, virus isolated; 0, virus not isolated.

hr, and at consecutive intervals from 144 through 216 hr. Previously frozen lung tissue (—60°) contained approximately 10^3 $TCID_{50}$ per 0.1 g lung tissue at 48 through 96 hr, and 10^1 at 24 hr. Lung tissue from animals harvested 120 hr postinoculation, and stored under these conditions, failed to demonstrate infectivity although the original cultures done prior to freezing contained virus. Virus was found in erythrocyte fractions obtained from animals at 4, 24, 48 and 120 hr after inoculation. Cultures of leukocytes and plasma from animals sacrificed at various intervals up to 504 hr after intranasal inoculation failed to show any virus.

Serologic response to infection. HI antibody titers of hamster plasma obtained prior to and at various intervals, up to 144 hr, following intranasal inoculation were $<1:10$. By 3 wk after inoculation, however, titers of 1:1280 were found.

Commercially prepared horse Para 3 antiserum (Flow Laboratories, Rockville, MD), tested simultaneously gave an HI titer $\geqq 1:2560$. The same commercially prepared horse antiserum gave neutralization titers $\geqq 1:80$. Simultaneously evaluated plasma from a group of 3 uninoculated control hamsters showed no neutralization at dilutions of 1:10. Hamster plasma from animals sacrificed 3 wk after intranasal inoculation of Para 3, showed a neutralization titer of only 1:10. Hamster plasma from animals sacrificed 3 wk following a second intranasal challenge of virus, (6 wk after the initial intranasal inoculation of Para 3), gave a neutralization titer of 1:20.

Discussion. The recovery of Para 3 from erythrocytes of hamsters, at intervals ranging from 4 to 120 hr after intranasal inoculation, during clinically inapparent pulmonary infection, represents the first report of viremia in this animal model. In addition to the repeated recovery of virus from blood and lung, Para 3 was isolated from liver on one occasion.

In previous attempts to demonstrate viremia with myxoviruses or paramyxoviruses, separation of blood into individual cellular and plasma components prior to inoculation into tissue culture, usually has not been done, or the reports contain no information as to whether individual components were tested (4, 6, 9, 12–15). It is said that viremia is rare, or that it does not regularly occur during myxovirus infections in man (16). There are only a few reports of influenza virus isolation from blood during natural or experimental respiratory infections (3, 13–15). However, in the case of influenza virus, viral particles are known to adsorb onto erythrocytes and platelets (17), to be adsorbed by lymphocytes, and to be phagocytosed by neutrophils (18). Also, erythrocytes with adsorbed virus are phagocytosed by leukocytes (19). In Colorado tick fever, intraerythrocytic virus has been noted during prolonged viremia (20). Recovery of virus from plasma or serum during viremia would not be anticipated if the particles were adsorbed to, or phagocytosed by cellular components of blood, and the chances of isolation would be relatively small if aliquots of whole blood were cultured while viral particles were concentrated in a single cellular component.

Isolation of hemadsorbing viruses from blood in tissue culture may also be influenced by such factors as phase of the illness, presence of serum antibody in the host, as well as whether samples have been frozen prior to tissue culture inoculation (3). In recent studies of viremia with hemadsorbing viruses, blood was often frozen prior to inoculation into tissue cultures (13, 15, 21). It has been a common experience in our laboratory that Para 3, even with the addition of 10% DMSO, cannot be re-isolated from specimens after freezing, especially when titers are quite low, such as probably occurs during Para 3 viremia. In addition, we have noted that calf serum, commonly used as medium for tissue cultures contains Para 3 HI antibodies, whereas fetal bovine serum does not. In these experiments, all original specimens were inoculated into tissue culture prior to freezing, with medium containing fetal bovine serum.

Not infrequently, a component of blood produces a toxic effect in tissue culture, thus interfering with recognition of viral specific CPE. Heparinized plasma from hamsters, guinea pigs, rabbits, rhesus monkeys and human subjects often produces morphologic alterations of varying extent in monolayer tissue cultures from various animal species. Electron micrographs of cell cultures incubated with plasma show branching, striated fibers with a periodicity and appearance similar to that of fibrin (22). It seems likely that the nonspecific CPE caused by whole blood or plasma is related to the production in tissue cultures of fibrin-like material. This nonspecific CPE can be inhibited by the addition of an excess amount of haparin and, nearly eliminated by washing tissue culture cells within 4 hr after the addition of plasma (22).

An effort was made to demonstrate viremia during attempted infection in animals previously immunized with intranasally administered Para 3, since some of our patients in whom Para 3 viremia has occurred have had significant levels of HI serum antibody (1). However, neither pulmonary infection nor viremia could be shown to occur following intranasal challenge with $10^{3.5}$ $TCID_{50}$ of Para 3 in animals inoculated intranasally 3 wk previously with 10^4 $TCID_{50}$ of this virus.

Summary. Parainfluenza Type 3 (Para 3) viremia has been demonstrated in hamsters by recovering virus from erythrocytes during pulmonary infection produced by the intranasal inoculation of virus. In attempts to isolate virus from blood, the plasma, erythrocytes and leukocytes were separated and cultured prior to freezing. Fetal bovine serum rather than calf serum was used in the tissue culture medium because the latter frequently contained Para 3 antibody. In addition, erythrocytes and leukocytes were allowed to remain in prolonged contact with tissue culture cells. The nonspecific CPE produced by plasma was avoided by washing tissue culture sheets 4 hr after the addition of plasma.

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