EXPERIMENTAL STUDY OF MIXED INFLUENZA – RS VIRUS INFECTION IN ALBINO MICE

V. V. Ritova, A. S. Larionov,
V. P. Moiseev, and V. V. Pshenichnikov

UDC 616.988.75-022.14-092.9-07

A mixed influenza–RS virus infection was produced experimentally in albino mice weighing 6-8 g by droplet and inhalation methods, and its nature was verified virologically and serologically. The course of the mixed virus infection was much more severe if the RS virus infection preceded the influenzal infection, produced by strain Hong Kong 68 of A2 influenza virus.

Few experimental studies have been made of mixed infections, although the need for such work is obvious from the results of epidemiological, clinical, and laboratory studies which have established the existence of acute mixed respiratory infections among children caused by association between strains of respiratory syncytial virus (RS) and the Hong Kong 68 strain of A2 influenza virus.

The object of this investigation was to study mixed influenza–RS virus infection in experiments on albino mice.

EXPERIMENTAL METHOD

Two types of virus strains were used in the present investigation. Strain Hong Kong 68 of A2 influenza virus was obtained from the virus museum of the Institute of Virology, Academy of Medical Sciences of the USSR; of the strains of RS virus, one (RS 43/1970) was isolated from a child in whom RS virus infection had been diagnosed, and the other, described as RS_{ad} (adapted), was reproduced in tracheal epithelial cells of albino mice and was isolated from them in the writers' previous investigation [1].

Altogether three series of experiments were carried out on 240 albino mice.

Sensitive strains of African green guenon kidneys (GGK) were used as cell cultures. The growth medium was a mixture of equal volumes of No. 199 and Eagle's medium with the addition of 0.1 mg L-glutamine per liter of medium and 10% calf serum. Antibiotics (penicillin, streptomycin, and nystatin) were added as usually recommended.

The maintainance medium consisted of a mixture of equal volumes of No. 199 and Eagle's medium with the addition of 2% inactivated calf serum.

For the serological investigations, immunofluorescence, the micromodification of the complement fixation test (CFT), the hemagglutination inhibition test (HIT), and the virus neutralization test in cell culture and in developing chick embryos as described in [2, 3] were used.

EXPERIMENTAL RESULTS

In two series of experiments (using the same scheme) the material was injected into one batch of animals (80 mice) intranasally under superficial ether anesthesia, and it was administered to another group

Ivanovskii Institute of Virology, Academy of Medical Sciences of the USSR. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR, V. S. Timakov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 72, No. 12, pp. 31-33, December, 1971. Original article submitted February 15, 1971.

TABLE 1. Titer of Complement-Fixing Antibodies in Sera of Albino Mice in CFT with 4 Units Antigen

Group of animals	RS virus, strain No. 43	Strain RS_{ad}	Strain Hong Kong 68 of A2 influenza virus
Batch I (inhalation of aerosols):			
1 – placebo	0	0	1 : 8
2 – RS_{ad}	0	1 : 64	1 : 8
3 – A2 Hong Kong 68	0	0	1 : 128
4 – mixed infection	1 : 64	1 : 64	1 : 64
Batch II (intranasal injection of material under ether anesthesia):			
1 – placebo	0	0	1 : 8
2 – RS_{ad}	0	1 : 128	0
3 – A2 Hong Kong 68	0	0	1 : 64
4 – mixed infection	1 : 8	1 : 256	1 : 64

TABLE 2. Determination of Antihemagglutinin Titer of Albino Mouse Sera

Group of animals	HIT with 4 units antigen	
	strain Hong Kong 68 of A2 influenza virus	association of RS_{ad} and Hong Kong 68 strain of A2 influenza virus antigens
Batch I (inhalation of aerosols):		
1 – placebo	20*	0
2 – RS	0	0
3 – A2 Hong Kong 68	640	80
4 – mixed infection	160	160
Batch II (intranasal injection of material under ether anesthesia):		
1 – placebo	0	0
2 – RS	0	0
3 – A2 Hong Kong 68	640	320
4 – mixed infection	320	320

*Reciprocals of titers of antihemagglutinins are given.

of animals (also 80 mice) by the aerosol inhalation method. Each batch of albino mice was divided into groups with ten animals in each group: 1) control, receiving a placebo consisting of maintainance medium of GGK cells cultivated for 5 days and not infected with virus; 2) receiving only the adapted strain of RS virus; 3) receiving strain Hong Kong 68 of A2 influenza virus; 4) receiving the adapted strain of RS virus and strain Hong Kong 68 of A2 influenza virus simultaneously.

After infection, the animals were kept at room temperature (20-22°C) and were observed daily. Death of the animals (two of ten mice) occurred only after mixed infection with the two viruses by the inhalation method, on the 4th and 6th days after infection, respectively. The supernatant of centrifuged suspensions of tracheal and lung tissues was tested for antigenicity. The surviving albino mice were totally exsanguinated and the serum collected and tested for the presence of antihemagglutinins and complement-fixing and virus-neutralizing antibodies.

TABLE 3. Times and Number of Dying Albino Mice after Intranasal Injection of Material under Ether Anesthesia

Material injected and scheme of injection	Time of death from moment of infection (in days)													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Mixed infection, simultaneous administration	0	0	0	1	1	1	0	0	0	0	0	0	0	0
Influenza virus 18 h after RS_{ad}	0	0	1	1	1	3	4	2	1	2	1	1	0	0
RS_{ad} 18 h after influenza virus	All mice of this group (20) remained alive throughout the period of observation (2 weeks).													
Placebo	All the albino mice were alive.													

The course of the disease was more severe in the albino mice infected with the material by the droplet method than by the inhalation method: death of an animal on the 5th day after infection was observed only when strain Hong Kong 68 of A2 influenza virus was given, while four of the ten mice died after mixed infection with the two viruses.

The results of serological investigation of the cooled sera from animals of each group of the two batches are given in Table 1. They show that the titer of complement-fixing antibodies was higher in mice infected with the association of influenza and RS viruses. In this case the droplet method of infection had no special advantage over the inhalation method. Correlation between the results was also found when hemagglutinins in the blood serum of the mice were tested (Table 2).

The results of titration of the virus-neutralizing antibodies in the blood sera of the albino mice were not so demonstrative as those of determination of the complement-fixing antibodies and antihemagglutinins and they are not described in this paper.

In the experiments of series III (80 animals), the scheme of the mixed infection was modified: the material was given by the droplet method intranasally under superficial ether anesthesia (Table 3).

The results given in Table 3 show that after simultaneous infection of the animals with an association of RS_{ad} virus and Hong Kong 68 strain of A2 influenza virus three mice died (each group in this series of experiments contained 20 animals). Meanwhile, a sharp increase in mortality among the animals occurred on the 6th-7th day after infection in the group of albino mice receiving the adapted strain of RS virus first by intranasal injection, followed after 18 h by the Hong Kong 68 strain of A2 influenza virus, but not in the reverse order. All the mice receiving the placebo, as well as in the control series, remained alive.

LITERATURE CITED

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