Acta virol. 27: 490—495, 1983

RESPIRATORY SYNCYTIAL VIRUS INFECTION IN SYRIAN HAMSTERS. I. DEVELOPMENT OF HUMORAL IMMUNITY

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Received May 18, 1982; revised December 16, 1982

Summary. — Adult Syrian hamsters have been shown useful for the study of respiratory syncytial virus (RSV) infection. Clinical manifestations coincided with the maximum replication of the virus in respiratory tract of infected animals as confirmed by immunofluorescence (IF) test. The dynamics of secretory and serum antibody responses is described in infected and reinfected animals.

Key words: respiratory syncytial virus infection; secretory and serum antibodies

Introduction

RSV is the most dangerous and widely spread causative agent of acute respiratory disease (ARD). The involvement of the lower respiratory tract was observed in 50% of infants in the 1st year of life and in 100% in the 2nd year (Parrott *et al.*, 1973). Although RSV infection has been investigated quite intensively since 1956, the development of local secretory and humoral immunity and its role in protection against infection still remain disputable. The study of the mechanisms of immune response is difficult because of the lack of a highly sensitive biological model. Signs of asymptomatic infection after intranasal infection swith RSV were observed in various animals: Syrian hamsters, cotton rats, and mice (Dreizin *et al.*, 1971; Leschinskaya *et al.*, 1972; Prince *et al.*, 1978, 1979). RSV was also demonstrated in the lungs of hamsters after intranasal infection (Zvereva *et al.*, 1973; Wallace, 1980).

The present study was aimed at investigation of the role of local secretory, humoral and cellular immune responses in Syrian hamsters infected with RSV.

Materials and Methods

Experiments were carried out in 120 adult hamsters weighing 100—120 g. Each hamster was inoculated intranasally under mild ether anaesthesia with 0.2 ml of the culture fluid containing 10^3 TID_{50}/ml of RSV (reference Long strain). Animals were infected by the same way and with

the same dose one month after primary infection. Control groups od hamsters received placebo (the liquid phase of uninfected HEp-2 cultures). To exclude contacts, the infected and unifected animals were kept separately.

Febrile reactions of animals were recorded daily with contact thermometer ET-2. Specimens for IF test (impression smears from trachea, lungs, spleen, liver, kidney and heart) and for serological examination (serum, tracheal washings) were collected at days 1, 3, 5, 7, 14, 20 and 30 after primary infection and reinfection. At each interval, 3—5 animals were used.

The specimens for the IF test were stained by the direct method using fluorescent antibodies to RSV prepared in the Laboratory of Diagnostic Preparation of the All-Union Influenza Research Institute of the USSR Ministry of Public Health (Mayboroda *et al.*, 1969). The brightness of fluorescence was assessed by the conventional 4-score method. As positive were considered preparations containing 5—7 morphologically typical intact cells with the brightness of fluorescence of at least degree 1+ in the nucleus or cytoplasm. The controls included impression smears of organs from the same animals stained with fluorescent antibodies to other viruses as well as preparations from uninfected hamsters.

To study the dynamics of secretory antibody production, tracheal washings were obtained at indicated intervals post-infection (p.i.) by a single washing of tracheas with 1 ml Eagle's medium. Tracheal washings were ultrasonicated for 30 sec at 700—750 nm before serological examination. Sera and tracheal washings were tested by indirect haemagglutination test (IHA) which is highly sensitive for detecting specific antibodies to RSV and not subjected to the effect of non-specific inhibitors (Rumel *et al.*, 1974). The IHA tests were performed in Takatsi microtitrator in panels with U-shaped wells. The results were read and recorded by the shape of the precipitate. All specimens obtained at different intervals after infection and reinfection were examined simultaneously with the same batch of the diagnostic preparation.

Results

All RSV-infected hamsters developed clinical manifestations of the disease. A significant increase in the body temperature was observed beginning from 3 days p.i. and reached the maximum (38.6 °C) at 7 days. By 13—14 days the temperature declined to normal (37.5 °C). During febrile reaction other clinical manifestations of the disease such as ruffled fur and aggressive behaviour were also observed. No clinical signs of disease were seen in the control group.

The period of fever coincided with the maximum replication of the virus in respiratory tract of infected animals as confirmed by results of IF tests (Table 1).

RSV could be detected in the trachea and lungs as early as 1 day p.i. The highest degree of specific fluorescence in these organs was observed 3 days p.i. which coincided with the onset of clinical manifestations of the disease. Later on the intensity of fluorescence in the trachea decreased rapidly so that at 5 days p.i. no RSV antigen could be detected, whereas in the lungs it was demonstrated up to 14 days p.i.

Apart from the lungs and trachea, the infectious process involved the spleen and liver. In the spleen RSV antigen was detectable up to 30 observation days. The intensity of immunofluorescence varied at different intervals; it was the highest at 5—7 days and the lowest at 1, 14, and 30 days p.i., respectively. In the liver, RSV antigen could be demonstrated only on day 5 p.i. No RSV antigen was detected in other organs (kidney, heart) examined at the same intervals. In the control group, the results of IF tests were negative.

Table 1. Results of IF examinations of organs from mature hamsters infected with RSV

Days of examination	Primary infection						Reinfection					
	lungs	trachea	spleen	liver	kidney	heart	lungs	trachea	spleen	liver	kidney	heart
1	+	+	−	−	−	−	+	+	+	−	−	−
3	+++	+++	++	−	−	−	++	++	++	−	−	−
5	+++	−	+++	+	−	−	++	++	++	−	−	−
7	+++	−	+++	−	−	−	−	−	−	−	−	−
14	++	−	++	−	−	−	−	−	−	−	−	−
20	−	−	+	−	−	−	−	−	−	−	−	−
30	−	−	+	−	−	−	−	−	−	−	−	−
Control	−	−	−	−	−	−	−	−	−	−	−	−

Antigen scoring: +, ++, +++;
no antigen: −.

Reinfection of the animals 30 days after primary infection was not accompanied by rise in temperature or by any other visible clinical manifestations. As confirmed by IF, reinfection of hamsters resulted in replication of RSV in the lungs, trachea and spleen for the first 5 days post inoculation. No RSV antigen could be detected in any other localization (liver, kidney, heart).

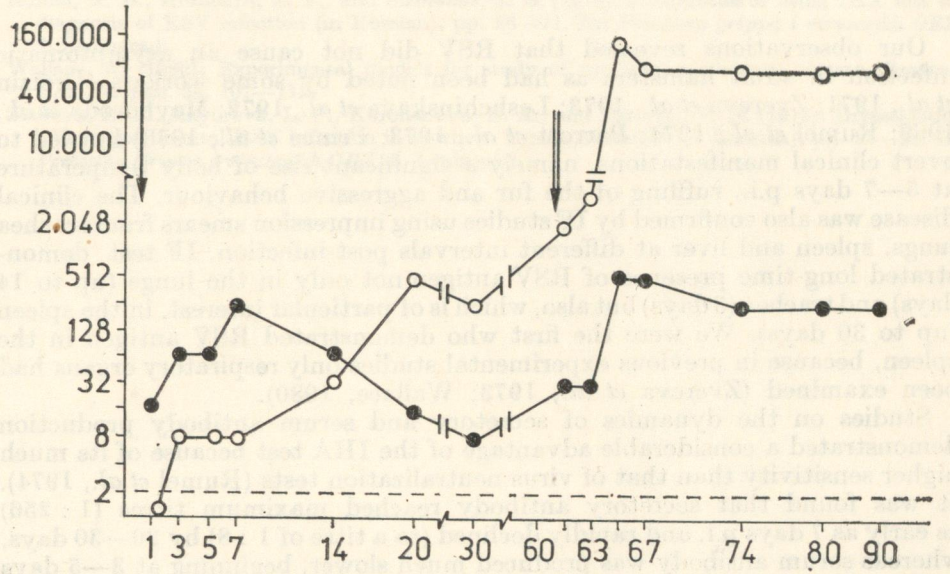

Fig. 1.

Dynamics of secretory and serum antibody production after infection and reinfection of Syrian hamsters with RSV

●——● secretory antibody titres, ○——○ serum antibody titres;
interrupted line:control.
↓ = primary infection (thin arrow)
⇓ = reinfection (wide arrow)
Abscissa: days after RSV infection and reinfection; ordinate: antibody titres.

The dynamics of local secretory and serum antibody response in primary infection and reinfection is shown in Fig. 1. As early as 24 hr p.i. a slight rise in specific secretory antibody (IgA) was observed, reaching the maximum titres at 7 days and declining to the minimal levels (1 : 8) by 30 days p.i. Reinfection of the animals at 30 days, i.e. in the period with minimal levels of secretory antibodies, caused more intensive antibody production reaching the maximum titres by the 5th day after reinfection. Simultaneously, the virus was eliminated from the hamsters as confirmed by the lack of demonstration in the organs tested at 7 days and later. At 30 days after reinfection, the level of secretory antibody was still sufficiently high. The

production of serum antibody showed a slightly different pattern. Maximum serum levels were reached by 20—30 days p.i. Reinfection led to a booster effect causing rapid and intensive serum antibody increase as early as 5 days after the secondary RSV administration; the high antibody levels remained unchanged for the 30 days observation period.

Discussion

Our observations revealed that RSV did not cause an asymptomatic infection in adult hamsters as had been noted by some workers (Dreizin *et al.*, 1971; Zvereva *et al.*, 1973; Leshchinskaya *et al.*, 1972; Mayboroda *et al.*, 1969; Rumel *et al.*, 1974; Parrott *et al.*, 1973; Prince *et al.*, 1978) but led to overt clinical manifestations, namely a significant rise of body temperature at 5—7 days p.i., ruffling of the fur and aggressive behaviour. The clinical disease was also confirmed by IF studies using impression smears from trachea lungs, spleen and liver at different intervals post-infection. IF test demonstrated long-time presence of RSV antigen not only in the lungs (up to 14 days) and trachea (3 days) but also, which is of particular interest, in the spleen (up to 30 days). We were the first who demonstrated RSV antigen in the spleen, because in previous experimental studies only respiratory organs had been examined (Zvereva *et al.*, 1973; Wallace, 1980).

Studies on the dynamics of secretory and serum antibody production demonstrated a considerable advantage of the IHA test because of its much higher sensitivity than that of virus neutralization tests (Rumel *et al.*, 1974). It was found that secretory antibody reached maximum titres (1 : 256) as early as 7 days p.i. and rapidly declined (to a titre of 1 : 8) by 20—30 days, whereas serum antibody was produced much slower, beginning at 3—5 days and reaching the maximum by 20—30 days p.i. The period of development of high serum antibody titres coincided with elimination of RSV antigen from the trachea and lungs. The animals were reinfected against the background of minimal levels of secretory and high levels of serum antibodies. At reinfection, there was no febrile reaction and IF examination revealed the presence of RSV antigen in the trachea, lungs and spleen only for 5 days after reinfection.

References

Dreizin, R. S., Vishnevetskaya, L. O., Bagdamyan, E. E., and Yankevich, O. D. (1971): Investigation of experimental RS virus infection in cotton rats. Virological and immunofluorescent studies (in Russian). *Vop. Virus.* **16**, 670—675.

Leshchinskaya, N. P., Zinzerling, A. V., Mayorova, L. P., Sekirina, G. G., and Suvorovova, E. A. (1972): Experimental infection of Syrian hamsters caused by respiratory syncytial virus and influenza virus (in Russian), pp. 101—109. In: *Problemy grippa i virusnykh ORZ, I*, Leningrad.

Mayboroda, G. M., Kamforin, L. E., and Borshchevsky, Yu. M. (1969): Methodic instructions for immunofluorescent method for diagnosis of influenza and other ARD of viral etiology (in Russian), pp. 7—19. In A. A. Smorodincev (Ed.): *Teoriya i praktika express diagnostiki grippa i ORZ metodom immunofluorescencii.* VNII Grippa MZ SSSR, Leningrad.

Parrott, R. H., Hyan, Wha Kim, Arrabio, I. O., Hodes, D. S., Murphy, B. R., Brandt, C. D., Camargo, E., and Chanock, R. M. (1973): Epidemiology of respiratory syncytial virus infection in Washington, D. C. II. Infection and disease with respect to age, immunologic status, race and sex. *Amer. J. Epidem.* **98**, 289—300.

Prince, G. A., Ienson, A., and Horswood, R. L. (1978): The pathogenesis of respiratory syncytial virus infection in cotton rats. *Amer. J. Pathol.* **93**, 771—791.

Prince, G. A., Berndt, J., Suffin, S. C., Horswood, R. L., and Chanock, R. M. (1979): Respiratory syncytial virus infection in inbred mice. *Infect. Immun.* **26**, 764—768.

Rumel, N. B., Korneeva, E. P., and Shtilbans, E. B. (1974): Possibilities of using IHA test for diagnosis of RSV infection (in Russian), pp. 66—71. In: *Problemy grippa i virusnykh ORZ*, 7, Leningrad.

Wallace, A. (1980): Experimental models for study of common respiratory viruses. *Environ. Hlth Perspect.* **35**, 107—112.

Zvereva, E. P., Mayorova, L. P., Kurchanova, E. A., and Parusov, V. N. (1973): Investigation of an experimental RSV infection in newborn Syrian hamsters (in Russian), pp. 93—98. In: *Problemy Grippa i virusnykh ORZ*, 8, Leningrad.