DYNAMICS OF LOCAL DEFENSIVE REACTIONS IN THE LUNGS IN ACUTE EXPERIMENTAL INFLAMMATION INDUCED BY RESPIRATORY SYNCYTIAL VIRUS

L. M. Voronina, T. N. Kop'eva, and O. V. Makarova

UDC 616.24-002.1-092.9-092:
612.017,1/-022:578 831.31

KEY WORDS: respiratory syncytial virus; lungs

The link between the character of an infectious process in the respiratory passages and the state of immune reactivity has received little study. There have been only isolated investigations into the effect of local and general immunity on the course of respiratory viral infection [2, 3, 6]. Nothing has been published in the accessible literature on changes in local immunity of the lungs under the influence of respiratory syncytial virus (RSV) infection, which accounts for 13.3% of all viral respiratory pathology in the adult population [6].

The aim of this investigation was to study the dynamics of local defensive reactions in the lungs of mice with acute experimental inflammation induced by RSV.

EXPERIMENTAL METHOD

Experiments were carried out on 60 male BALB/c mice weighing 14-16 g and aged 18 weeks. Of this number 42 mice were infected with 0.1 ml of a culture of RSV ($5 \cdot 10^2$ PFU/ml). Animals of the control group (18 mice) were given 0.1 ml of culture medium not containing virus by the intranasal route. The titer of RSV antigen in the blood was determined by ELISA [5]. The animals were killed by cervical dislocation on the 6th, 9th, 14th, 26th, and 32nd days of the experiment, seven mice in each group. The right lung was used for histological study, the left to obtain cells by the enzymic elution method [4]. A semiquantitative estimation of the state of the bronchus-associated lymphoid tissue (BALT) was undertaken on the histological sections and the number of lymphocytes and histiocytes was counted in foci of concentration: normal — under 15 cells, mild hyperplasia 16-25 cells, moderate hyperplasia 26-50 cells, severe hyperplasia — over 50 cells. RSV antigen was discovered in frozen sections by the direct immunofluorescence method using labeled immunoglobulins to RSV. Bacterioscopic investigation of the tissues was carried out on serial paraffin sections stained by the Gram—Weigert method. The absolute number of cell in lung tissue eluates and their viability were determined in a Goryaev counting chamber. Suspensions of cells with viability of over 88% were used. The cytology of the eluates was determined quantitatively in films of cell suspension stained by Romanovsky's method. Tests of chemotaxic and phagocytic activity were carried out on suspensions enriched with neutrophils and mononuclears [1]. Phagocytic activity of the macrophages was estimated with the aid of a 24-h culture of *E. coli* 675 in a suspension with a concentration of $2 \cdot 10^6$ cells/ml. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

On the 6th-9th days of the experiment the appetite of the experimental mice was reduced, they developed conjunctivitis, and became immobile On the 14th-19th days, the animals' hair appeared dull and untidy, and the mice hid in the

Laboratory of General Pathological Anatomy, Research Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 5, pp. 512-515, May, 1991. Original article submitted September 22, 1990.

 0007-4888/91/0005-0666$12.50

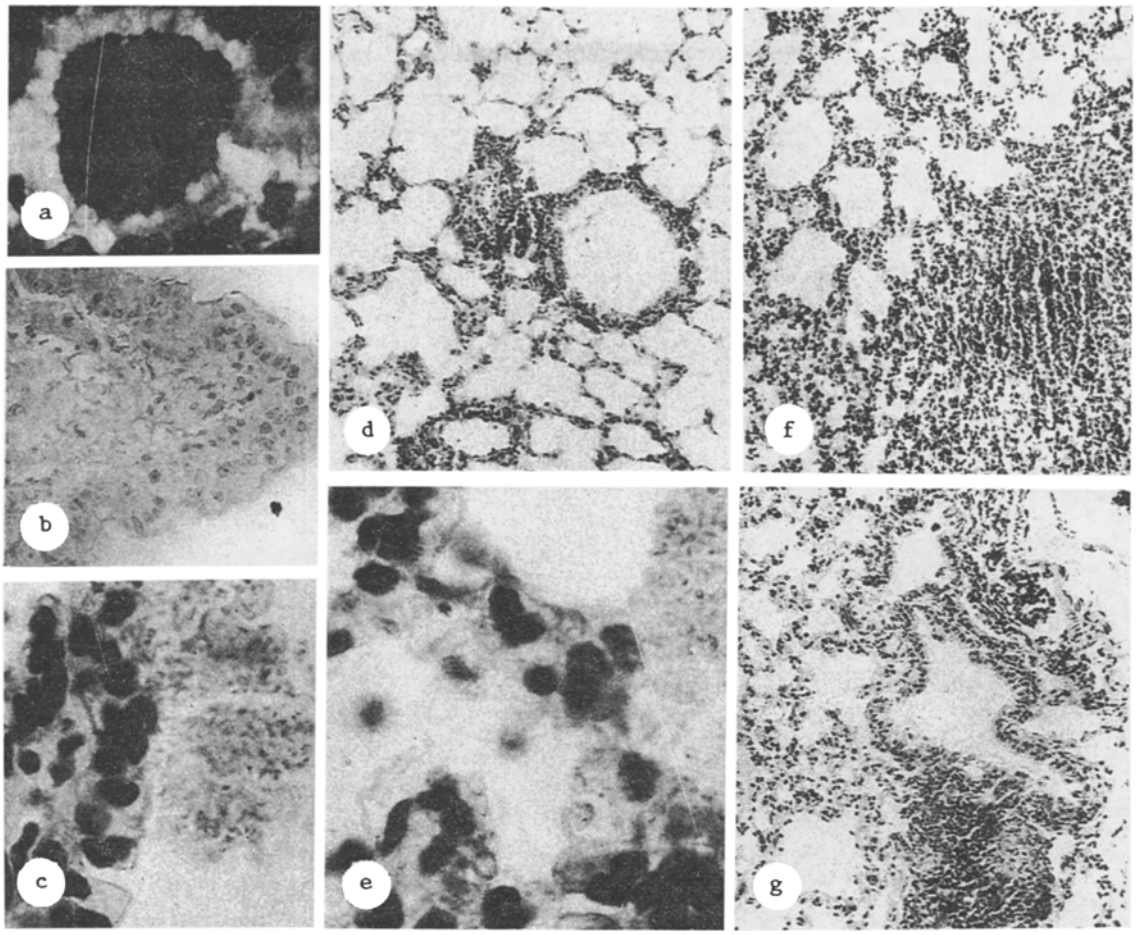

Fig. 1. Morphological changes in lungs in acute inflammation induced by RSV. Sixth day of experiment. Viral bronchitis. a) Focal luminescence of RSV antigen in epithelium of a bronchiole. Direct Coons' method. 400×; b) vacuolation of cytoplasm and anisonucleosis of epithelium, congestion of capillaries, stratification of epithelium with syncytium formation. Semithin section. Toluidine blue. 400×; 9th day of experiment; c) colonies of bacteria on cilia of epithelium of respiratory bronchiole. Gram—Weigert stain. 900×. Oil immersion. 14th day of experiment; d) moderate hyperplasia of BALT at level of noncartilaginous bronchus. Hematoxylin and eosin. 200×; e) colonies of bacteria on surface and in wall of interalveolar septum; inflammatory infiltration. Gram—Weigert stain. 900×. Oil immersion 19th day of experiment; f) acute pneumonia: exudate in lumen of alveoli. Region of abscess formation with destruction of interalveolar septa. Hematoxylin and eosin 200×. 26th day of experiment; g) stage of resolution of acute inflammation in lungs. Severe hyperplasia of BALT at level of noncartilaginous bronchus. Hematoxylin and eosin. 200×.

corner of the cage. They started to become more active again on the 26th day. By the 32nd day the animals had regained their usual appearance, but still remained apathetic to some degree. The titer of RSV antigen in the blood of the mice was maximal (1:256) on the 6th day of the experiment, it decreased (1:16) on the 9th day, and was indistinguishable from values in the control group on the 14th-42nd days (1:4). Immunofluorescence and virologic investigations revealed RSV antigen in the epithelium of the small bronchi and bronchioles on and after the 6th day (Fig 1a). Macroscopic changes in the lungs in the form of foci of pneumonia were detected on the 9th-26th day of the experiment.

Histological investigation of the lungs of the mice on the 6th day of the experiment revealed viral bronchitis. Starting from the level of the segmental bronchi and as far as the respiratory bronchioles, degenerative changes were found in the epithelial cells in the form of vacuolation and anisonucleosis, focal desquamation and stratification of the epithelium, and syncytium formation (Fig. 1b). Scanty infiltration with neutrophils was observed in the lamina propria of the mucosa

TABLE 1. Cell Composition and Functional Activity of Lung Tissue Eluates from Mice Infected with RSV and Control Group

Parameter	Control group	Experimental group, on undermentioned day of experiment			
		6-th	9-th	14—19-th	26—32 th
Macrophages					
%	13,30±0,28	13,00±2,14	15,00±1,01	6,30±0,04*,**	21,90±0,12*,**
absolute	0,46±0,01	0,28±0,01*	0,31±0,04*	0,91±0,08*,**	1,40±0,76
Lymphocytes					
%	0,80±0,01	1,00±0,02*	3,00±0,10*,**	12,55±0,49*,**	24,15±0,40*,**
absolute	0,02±0,001	0,02±0,01	0,06±0,01*,**	2,27±0,22*,**	3,26±0,20*,**
Neutrophils					
%	0,96±0,002	1,50±0,02*	4,00±0,04*,**	6,02±0,15*,**	9,21±0,53*,**
absolute	0,03±0,01	0,02±0,01	0,08±0,01*,**	1,03±0,09*,**	0,28±0,01*,**
Tissue cells					
%	82,71±4,38	84,50±6,18	78,01±8,01	72,15±0,81	59,16±0,61*
absolute	2,91±0,26	1,34±0,01*	1,64±0,66*,**	9,75±0,78*,**	7,56±0,44*,**
Chemotaxis, μ:					
neutrophils	8,14±0,30	9,60±0,51*	7,20±0,40**	6,11±0,04*,**	9,10±0,07*,**
macrophages	9,20±0,37	8,30±0,42	15,30±0,51*,**	9,81±0,09**	11,35±0,92**
Phagocytosis of macrophages					
index of attraction	236,01±3,30	265,01±7,60*	470,02±6,80*,**	550,01±4,38*,**	357,50±7,91*,**
phagocytic number, %	93,00±2,60	85,00±3,60	100,00±0,00*,**	92,51±0,34**	80,01±0,31*,**
phagocytic index	3,33±0,40	3,30±0,81	5,15±0,90	5,25±0,10*	4,63±0,07*,**

Legend. Significance of differences ($p < 0.05$): *) compared with control, **) with previous group.

and the epithelium. BALT did not differ from the control. Bacterioscopic examination of the tissues showed that the composition of the microflora and its prevalence at different levels of the bronchial tree were the same as in the control. Bacteria were represented by Gram-positive cocci, which were discovered on the cilia of the epithelium and in the secretory layer of single large bronchi (from 2 to 6 per field of vision under magnification of 900). On the 9th day of the experiment desquamation of the epithelium, edema, and inflammatory infiltration of the bronchial mucosa increased, and slight hyperplasia of BALT was observed, The number of microorganisms colonizing the cilia of the epithelium (Fig. 1c) and in the composition of the exudate in the large and small bronchi increased significantly (15-100 per field of vision). On the 14th-19th days of the experiment focal pneumonia and moderate hyperplasia of BALT developed (Fig. 1d-f). Bacterioscopic examination of the tissues revealed numerous polymorphic bacteria (100-150 per field of vision) in the bronchial wall, exudate, and foci of abscess formation with acute pneumonia. On the 26th-32nd day of the experiment, single foci of resolved pneumonia and atalectasis still remained in the lungs of the mice, but hyperplasia of BALT was severe (Fig. 1g). The character and distribution of the microflora were the same as in the control.

The cytological composition of the lung tissue eluates and functional activity of the phagocytes in the course of the inflammatory process are shown in Table 1. In the period of viral inflammation, the number of macrophages in lung tissue eluates was reduced. Changes in their functional activity were limited to an increase in their attractive ability. A significant increase in chemotaxic activity of the neutrophils also was found, but their number was not increased. In the period of mixed viral-bacterial infection the number of macrophages remained below normal, but the numbers of neutrophils and leukocytes increased significantly. At this time the chemotaxic activity of the neutrophils declined but, conversely, parameters of chemotaxis and phagocytosis of the macrophages increased. The period of acute bacterial inflammation was characterized by a further increase in the number of lymphocytes and neutrophils, and also by a significant increase in the number of macrophages. The chemotaxic activity of the phagocytes was depressed. As regards the phagocytic activity of the macrophages, their attractive capacity continued to increase whereas the number of phagocytic cells decreased. In the period of convalescence from inflammation the number of neutrophils was significantly lower than in the phase of bacterial inflammation, although it remained higher than the control values. The absolute and relative numbers of macrophages and lymphocytes also remained high. Functional activity of the phagocytes was characterized by higher values of chemotaxis of neutrophils and macrophages than in the control. The phagocytic activity of the macrophages decreased but the index of attraction remained above normal.

Thus the particular features of the local defensive reactions in the lungs in acute inflammation depend on the character of infection. In viral inflammation in the lung tissue, a decrease in the number of macrophages is found, together with an increase in their powers of attraction, and in the chemotaxic activity of the neutrophils. If a bacterial flora is present as well, the numbers of neutrophils, lymphocytes, and macrophages increase, and moderate hyperplasia of BALT develops. Functional activity of the lung phagocytes is characterized by weakening of chemotaxis of the neutrophils and strengthening of the phagocytic capacity of the macrophages. In the phase of convalescence the number of neutrophils decreases, the number of lymphocytes and macrophages remains high, and hyperplasia of BALT increases in severity. Functional activity of the phagocytes remains at higher levels than normally.

LITERATURE CITED

1. S. D. Douglas and P. G. Quie, Investigation of Phagocytoses in Disease, Edinburgh (1981).
2. I. V. Pokhodzei, O. A. Sukhovskaya, and V. D. Kulikov, Immunologiya, No. 5, 65 (1988).
3. N. V. Putov, N. V. Yakovleva, I. V. Pokhodzei, et al., Ter. Arkh., No. 3, 49 (1990).
4. J. Baert, Acta Anat. (Basel), **136**, 89 (1989).
5. S. L. Epstein and I. K. Lunney, J. Immunol. Meth., **76**, 65 (1985).
6. H. H Zakonkian and I. Leader, Ann. Intern. Med., **109**, No. 6, 515 (1988).

ENZYME ACTIVITY OF THE INTERFERON SYSTEM IN VIRUS DISEASES

T. M. Sokolova, I. A. Suetina, N. N. Nosik, and V. F. Krylov

UDC 616.98:578.8]-085.355 578.245

KEY WORDS: enzymes of the interferon system; virus diseases

The interferon system plays a definite role in resistance of the individual to viral infections on the first days of the disease and until the appearance of specific antibodies [9, 16, 17]. Many known viruses induce interferon in the body, and for that reason raised levels of circulating interferon are found in the blood in acute viral infections, but these quickly disappear. The antiviral action of interferon is due to activation of two enzymes in the cells, namely 2′,5′-adenylate svnthetase and a specific protein kinase [6, 8]. Since interferon-dependent enzymes have proved themselves to be stable markers of interferon action, their determination has come to be widely used in clinical studies [5, 11]. Activation of the enzymes has been demonstrated after injection of exogenous natural and recombinant interferons, after vaccination, and in acute virus diseases [10, 15]. Previously we studied the interferon status and activity of enzymes of the interferon system in individual virus diseases [1, 2]. In the investigation described below, changes in the enzymes in virus diseases of different etiology and with different clinical course has been compared for the first time: influenza, complicated by pneumonia, parainfluenza, chronic hepatitis B with delta-infection, and recurrent urticaria with frequent acute respiratory virus infection (ARVI) and herpes. Tests for interferon-dependent enzymes were used for the first time also to assess the effectiveness of treatment of virus diseases with recombinant α_2-interferon.

Laboratory of Viral Ontogeny, D. I. Ivanovskii Institute of Virology, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR D. K. L'vov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 111, No. 5, pp. 515-516, May, 1991. Original article submitted July 24, 1990.