USE OF A MODEL OF EXPERIMENTAL PNEUMONIA FOR THE COMPARATIVE STUDY OF THE PATHOGENICITY OF *Mycoplasma pneumoniae*

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The agent causing primary atypical pneumonia was first described as an infectious agent of virus nature [5], and not until 18 years later was it shown that it is in fact not a virus but a member of the family Mycoplasmatacae [2], subsequently named Mycoplasma pneumoniae [3].

The possibility of reproducing a model of this infection was first demonstrated by Eaton in 1944, who inoculated cottontail rats aged 6-8 weeks intranasally with the pharyngeal washings of patients with primary atypical pneumonia. In this way pneumonia was reproduced irregularly, developing in approximately half the animals used in the experiment. In subsequent investigations this method was used to study the action of antibiotics on the agent of this disease [7-8], to investigate the neutralizing activity of antisera [6], and for other purposes. The infection was reproduced successfully in volunteers [1]. In a recent paper [4], the results of human infection with the laboratory strain FH of M. pneumoniae the pathogenicity of which was depressed by prolonged passage in artifial nutrient media, were described. After inoculation, in some of the volunteers the temperature rose although pneumonia did not develop. It was shown, however, that in 83% of infected persons the antibody titer reached a level adequate for preventing the development of the disease in case of natural infection. The authors of this paper raise the question of the possibility of creating attenuated strains for the immunoprophylaxis of natural atypical pneumonia in man caused by M. pneumoniae.

The object of the present investigation was to reproduce an experimental infection caused by the attenuated laboratory strain FH of M. pneumoniae and to attempt to discover the possible indices of pathogenicity for other strains of mycoplasmas of this species.

EXPERIMENTAL METHOD

To produce the experimental infection, cottontail rats were inoculated intranasally with different doses of viable particles of laboratory strain FH of M. pneumoniae, obtained from Dr. R. M. Chanock (United States), and of strain No. 62 isolated by the authors in Moscow from a patient with primary atypical pneumonia and identified by the β-hemolysis test as M. pneumoniae. The animals of the control group were inoculated intrnasally with washings from a sterile nutrient medium. The rats were sacrificed at various times after inoculation, and the character and intensity of the inflammatory changes in the blood tissue were first assessed macroscopically. Later the tissue was fixed, histological preparations were made, and these were examined under the microscope.

EXPERIMENTAL RESULTS

In the experiments of series I experimental pneumonia was reproduced in cottontail rats aged 6-8 weeks. In the experiment more than 100 rats of this age were inoculated with strain FH of M. pneumoniae in doses of between 500,000 and 2 million viable particles. However, in these experiments no lesions of the lung tissues visible with the naked eye were obtained. As a control for the experiments with the laboratory strain FH, cottontail rats aged 6-8 weeks were inoculated intranasally with strain No. 62 of M. pneumoniae recently isolated from a patient. The results of this experiment showed that the virulent

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TABLE 1. Results of Reproduction of Experimental Pneumonia by Intranasal Inoculation of Newborn Rats with Laboratory and Recent Strains of M. pneumoniae

Dose (in viable units)		Strain FH			Strain No. 62				Control (no. inoculation with infected material)
		250,000	25,000	2,500	250,000	25,000	2,500	250	
No. of animals		16	15	16	16	16	14	15	14
Character of macroscopic changes	Total bronchopneumonia	–	–	–	2	–	–	–	–
	Lobar bronchopneumonia	1	–	–	5	2	–	–	–
	Large foci of pneumonia	3	–	–	4	4	–	–	–
	Solitary small foci of pneumonia	3	2	–	2	1	4	–	–
	Absence of macroscopic changes	9	13	16	3	9	10	15	14

TABLE 2. Dynamics of Development of Macroscopic Changes in the Lungs of Newborn Rats during Experimental Reproduction of Pneumonia by Laboratory Strain FH of M. pneumoniae (Intranasal Inoculation in a Dose of 750,000 Viable Particles)

Character of macroscopic changes	Time after inoculation (in days)					
	1st	2nd	5th	10th	20th	30th
	No. of animals					
	18	20	17	16	18	18
Total bronchopneumonia	–	–	1	1	–	–
Lobar bronchopneumonia	1	1	5	1	–	–
Large foci of pneumonia	1	3	1	4	–	1
Single small foci of pneumonia	4	3	2	5	5	–
Absence of macroscopic changes	12	13	8	5	13	17

strain No. 62, in the same doses and experimental conditions, could produce macroscopic changes in the lung tissue in more than half the animals. Histological investigation of this material showed that on the 6th-7th day after inoculation with strain No. 62, in nearly all cases foci of bronchopneumonia of varying size were observed, in some places serous and desquamative, in others suppurative and desquamative in character. A distinctive feature of the experimental infection caused by strain No. 62 was the presence of productive vasculitis in many animals, represented mainly by cells of the lymphoid series with an admixture of leukocytes. The vessel walls were usually soaked with plasma. In addition to the vasotoxic effect just described, frequently hemorrhages were found in the lumen of the alveoli, with signs of hemolysis. Around the bronchioles considerable areas of lymphocytic infiltration and circumscribed collections of large cells of desquamated epithelium were found.

The results of these experiments showed that strain FH of M. pneumoniae possesses lowered virulence and cannot cause the development of a well marked infectious process in rats aged 6-8 weeks. For this reason, in the attempt to reproduce the infection and determine the residual pathogenicity of this strain, a series of experiments was carried out in which newborn cottontail rats were inoculated intranasally (Table 1).

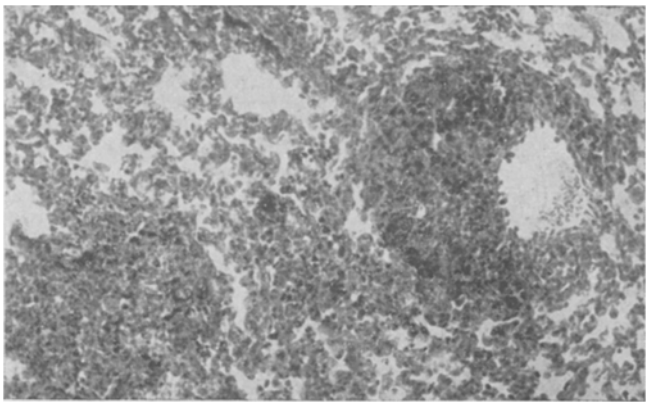

Fig. 1. Changes in the lung tissue on the 7th day after intranasal inoculation of newborn rats with the recently isolated strain No. 62 of M. pneumoniae in a dose of 250,000 viable particles. Marked vasculities against a background of serous-desquamative pneumonia. Hematoxylin-eosin. 100×.

It is clear from Table 1 that the strain FH caused macroscopically visible pneumonia in almost half (in 7 of 16) the animals used in the experiment. Meanwhile strain No. 62, in the same doses, produced lesions which were far more intensive and which occurred in 13 of the 16 infected rats. When comparing the nature of the histological changes produced by these two strains in the newborn rats, it should be noted that in infection with strain FH, large and small foci of pneumonia were found in different parts of the lung and were serous and desquamative in character, sometimes with a considerable admixture of leukocytes. Foci of peribronchial pneumonia were seen everywhere, consisting mainly of lymphocytes. Usually pneumonia was found against the background of severe congestion of the lungs and extensive hemorrhages in the lumen of the alveoli. In the newborn rats inoculated with the recently isolated virulent strain No. 62, extensive foci of mainly serous and desquamative pneumonia were also found, but in contrast to infection with the laboratory strain FH, in all cases areas of specific vasculitis also were found (Fig. 1). The wall of the blood vessels was saturated with plasma. Such vessels, of arteriolar type,

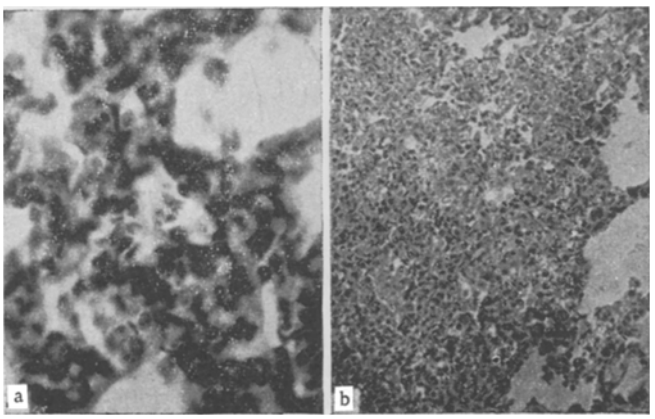

Fig. 2. Changes in the lung tissue after intranasal inoculation of newborn rats with laboratory strain FH of M. pneumoniae in a dose of 750,000 viable particles. a) First few hours after inoculation: interstitial pneumonia, marked hyperplasia of nuclei of the cells of the interalveolar septa, admixture of leukocytes. Hematoxylin-eosin. 200×; b) toward the 7th day after inoculation: foci of sero-desquamative pneumonia, well marked desquamation of cells of the alveolar epithelium. Hematoxylin-eosin. 100×.

were usually accompanied by bronchioles, so that the cuffs of lymphocytes surrounding the vessel also spread to some extent to the peribronchial tissue. Staining of the elastic tissue confirmed the perivascular situation of the infiltration. The elastic skeleton in these circumstances appeared swollen. In some cases, a large admixture of leukocytes was present in the areas of infiltration around the blood vessels.

The study of the development of pneumonia caused in the newborn rats by intranasal inoculation with 750,000 viable particles of strain FH of M. pneumoniae showed (Table 2) that macroscopically visible changes in the lung tissue were present in some animals on the 1st and 2nd days after inoculation. On the 5th-10th day in most rats used in the experiment, the inflammatory changes were maximal. By the 30th day a focus of pneumonia could be found in only one of the 18 rats. Histological examination showed that the initial reactive changes developed in the lungs during the first few hours after inoculation, and consisted mainly of reactions of vascular type, in the form of congestion, stasis, and small foci of interstitial pneumonia (Fig. 2a). The exudative reaction at this time was slight, but it progressed steadily and by the end of the first day a sero-desquamative and sero-purulent pneumonia had developed on a considerable scale. The inflammation developed against the background of thrombosis of blood vessels and disturbance of the circulation. These phenomena increased in severity and reached a maximum on the 5th-10th days after inoculation (Fig. 2b). In the later periods the picture of resolution of the process was observed, and by the 30th day no inflammatory changes were usually present in the lung.

In the same experiment the rate of survival of the agent in the body of the newborn rats was studied. A gradual decrease in positive seeding of M. pneumonia from the lungs of sacrificed animals was observed, and in some cases (on the 10th-20th-30th days after inoculation) there was no clear correlation between the considerable macroscopic changes in the lung tissue and the positive result of seedings on nutrient media. On the 30th day, M. pneumoniae was isolated from the lungs of 5 of the 18 sacrificed rats.

The results of the further study of the age resistance of rats to intranasal inoculation with strain FH of M. pneumoniae showed that their sensitivity to this infection diminished gradually with age. For example, whereas during infection of young rats during the first week of life, depending on the dose of infecting material, pneumonia could be produced in 50 or 67% of the animals used in the experiment, in the case of rats aged 3 weeks this proportion fell to 25-20%, and in animals aged 6 weeks, as shown above, practically no macroscopic signs of pneumonia were produced.

The results described above thus show that, depending on their age, cottontail rats differ in their resistance to experimental infection caused by various strains of M. pneumoniae. This phenomenon can be used to determine the pathogenicity of strains of M. pneumoniae. Experimental reproduction of the infection in newborn rats is most advisable, because it is only in these conditions that the level of pathogenicity, not only of recently isolated, but also of attenuated laboratory strains, can be detected. In the experimental reproduction of the infection, one of the indices of pathogenicity of M. pneumoniae is evidently the property of forming the characteristic lesions of vasculitis.

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