Mycoplasma hominis AS AN AGENT OF DISEASES OF THE RESPIRATORY TRACT

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The respiratory organs of newborn rats infected intranasally with a broth culture of *Mycoplasma hominis* were studied by histological, histochemical, and immunofluorescence methods. Tracheitis and suppurative and interstitial pneumonia with a hemorrhagic component were observed 24 h after infection and lasted until the 7th day of the experiment. During the same period, specific fluorescence of *M. hominis* antigen was detected by the immunofluorescence method. The morphological picture found demonstrates the pathogenicity of *M. hominis* for the respiratory tract of newborn rats.

KEY WORDS: Mycoplasma hominis; *pneumonia.*

The wide distribution of acute respiratory diseases in man at all ages is a stimulus for the search for the agent causing these diseases. In the last decade reports have been published [8, 9] that if the urogenital mycoplasma (*Mycoplasma hominis*) is introduced into the respiratory tract of volunteers, some of them develop pharyngitis and the presence of infection is confirmed in nearly all of them by a fourfold increase in the antibody titer in the indirect hemagglutination test. In publications by Clyde et al. [6] and Hendley et al. [7] it is stated that *M. hominis* is associated with the development of pharyngitis in children and adults. Bashmakova and Soldatova [3] and Bashmakova [1, 2] found a picture of marked bronchopneumonia during a histological investigation of fetuses from whose lungs *M. hominis* was isolated. Pagava and Goncharova [5] found an increase in the antibody titer against *M. hominis* by the indirect agglutination test in children aged from 2 weeks to 4 years with various respiratory diseases. There is thus weighty evidence of the pathogenicity of *M. hominis* for the respiratory tract. However, the only research in this direction has been the morphological study of Gusman et al. [4], in which in albino rats and guinea pigs infected either by introduction of a culture into the conjunctiva or by intratesticular injection, the animals developed pneumonia characterized by a marked plasma-cell reaction, conjunctivitis, and degenerative changes in the internal organs.

The object of the present investigation was to study whether respiratory infections can be produced by infecting cotton-tail rats with *M. hominis*.

EXPERIMENTAL METHOD

Cotton-tail rats aged 2-3 weeks were infected intranasally and nasopharyngeally under superficial hexobarbital anesthesia by instillation of 0.1 ml of a broth culture of *M. hominis* in a titer of $1 \cdot 10^6$ CFU/ml. Uninfected animals and also young rats into which pure broth medium was instilled served as the control. The experimental animals were decapitated 2-3 h and 1, 2, 3, 4, 7, 11, 14, 21, and 28 days after infection.

To detect *M. hominis* in the tissues of the trachea and lungs these organs were cultured and squash preparations from them were tested by the indirect Coons' immunofluorescence method. The respiratory organs (trachea and lungs) of the experimental animals were studied

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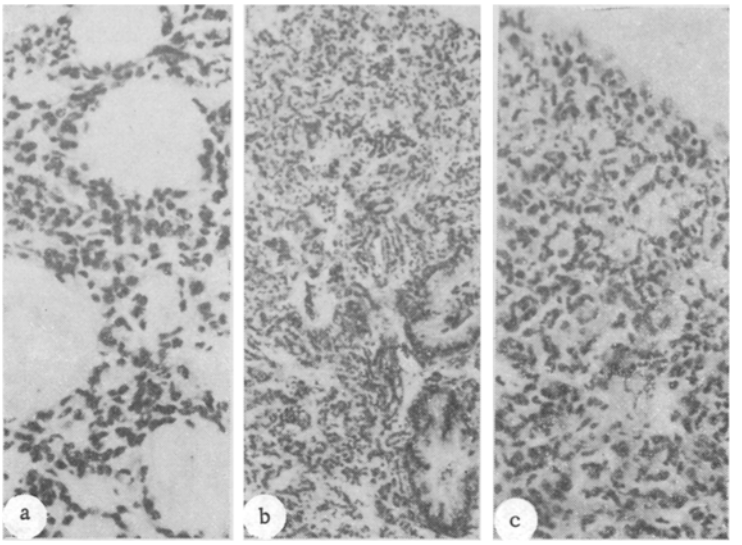

Fig. 1. Lung 24 h after intranasal infection with *M. hominis*. a) Foci of interstitial pneumonia (250 ×); b) foci of desquamative-suppurative pneumonia (120 ×); c) cellular infiltration of pleura (250 ×). Hematoxylin–eosin.

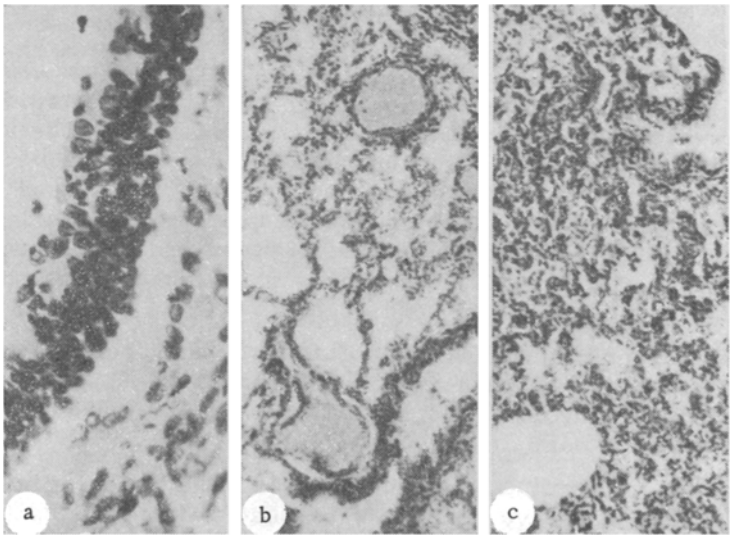

Fig. 2. Changes in lungs on 2nd day after infection with *M. hominis*. a) Edema and infiltration by leukocytes of epithelial and subepithelial layers of trachea (500 ×); b) thrombi in vessels, hemorrhage into lumen of alveoli (120 ×); c) focus of bronchopneumonia with destruction of bronchial wall (120 ×). Hematoxylin–eosin.

by histological (hematoxylin–eosin, Van Gieson's method) and histochemical (Brachet's, Feulgen's, and PAS reactions, impregnation with silver by Gomori's method) methods.

EXPERIMENTAL RESULTS

In the respiratory organs of rats infected intranasally, edema of the epithelial and subepithelial layers was found on the 1st day after injection of the culture of *M. hominis* in the trachea, and large quantities of PAS-positive round formations of different sizes were discovered in the cytoplasm of individual epithelial cells by the PAS reaction (antigen of *M. hominis*). In the lungs, on the 1st day, dilation and congestion of the blood vessels

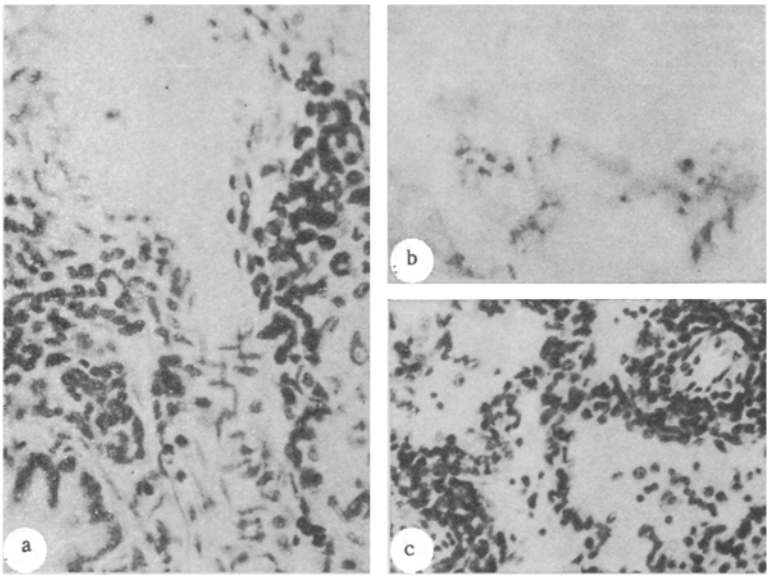

Fig. 3. Changes in lungs on 7th day after injection of *M. hominis*. a) Plasma cells in perivascular and peribronchial tissue (Brachet's reaction; 250 ×); b) mycoplasmas in desquamated alveolocytes in lumen of alveoli (PAS reaction, immersion); c) perivascular infiltration with monocytes (hematoxylin–eosin, 120 ×).

were found. The endothelium and walls of the blood vessels were swollen, with accumulation of PAS-positive material in them. The epithelium of the bronchi was edematous and contained PAS-positive material, which was desquamating in some places. Solitary leukocytes were found in the lumen of the bronchi. The PAS reaction revealed the antigen of *M. hominis* in the lumen of the bronchi and alveoli, in the desquamated alveolocytes, and also extracellularly. Edema of the alveolar septa was very conspicuous and the septa themselves were thickened over a wide area as the result of infiltration by lymphocytes and leukocytes. Small and large areas in which the lumen of the alveoli were packed with leukocytic exhudate containing small numbers of desquamated alveolocytes also were present.

During the 1st day after intranasal infection of young rats, a widespread interstitial (Fig. 1a) and focal desquamative-suppurative bronchopneumonia (Fig. 1b) could thus be discovered. The pleura also was involved and showed edema and diffuse cellular infiltration (Fig. 1c), in which there were many leukocytes.

On the 2nd day after infection the intensity of the pathological process increased. In the trachea edema and degenerative changes were observed in the epithelial layer, with focal desquamation and infiltration of that layer by leukocytes; edema and cellular infiltration were observed in the submucous layer (Fig. 2a). The PAS reaction revealed PAS-positive granules on the surface of the epithelial cells. The circulatory disorders in the lungs were increased in intensity, sometimes with the formation of thrombi and hemorrhages in the lumen of the alveoli (Fig. 2b), desquamation of the epithelium of the bronchi and alveolocytes, and severe lesions of the bronchi with destruction of their walls (Fig. 2c). Otherwise the picture was the same as that observed at the previous period, but the PAS reaction showed antigen of *M. hominis* mainly inside the desquamated alveolocytes.

On the 3rd day after infection the process in the lungs was less acute: Fewer leukocytes were present in the exudate and the foci of bronchopneumonia were less extensive; metaplasia of the epithelium was observed in the bronchi, and PAS-positive mycoplasmas were present in it. In the trachea, besides the changes already described above, evidence was found of metaplasia of the epithelium and foci of lymphocytic infiltration into the subepithelial layer, which contained many mast cells. By the 4th day only tiny foci of desquamative and interstitial pneumonia could be seen in the lungs. In the periobronchial follicles at this time mast cells appeared. The PAS reaction revealed mycoplasmas in the lumen of the alveoli, where they were free-lying or located in desquamated alveolocytes (Fig. 3b). The

picture of an inflammatory process still continued in the trachea. Starting from the 7th day and until the end of the experiment (28th day) the picture in the lungs of the infected animals was the same as that in the control except for the appearance of plasma cells, mainly in the perivascular and peribronchial tissues (Fig. 3a). The number of plasma cells increased as the duration of the experiment was lengthened, indicating the development of immunological reactions. An acute condition was found in one animal on the 11th day, namely macrofocal bronchopneumonia with few leukocytes in the exudate and a diffuse interstitial reaction with the formation of large areas of perivascular monocytic infiltration (Fig. 3c). The picture discovered is evidence of exacerbation of the process associated with sensitization of the animal. The absence of a bacterial flora on staining by the Gram–Weigert method indicates that the exacerbation of the process was due to *M. hominis*.

At all periods of investigation foci of emphysema were observed, with solitary large formations of different sizes, rich in DNA, in the alveolar septa. These were evidently conglomerates of degenerated nuclei. Starting from the 7th day, besides desquamation of the epithelium, proliferation with the formation of papillary growths was observed in the bronchi. At all periods of the experiment the PAS reaction revealed antigen of *M. hominis*, although specific fluorescence during the immunofluorescence test was detected only until the 7th day.

The morphological study of the respiratory organs of young rats aged 2-3 weeks, infected intranasally by the urogenital mycoplasma *M. hominis*, thus demonstrated its pathogenicity for the respiratory tract, as shown by the development of suppurative and interstitial pneumonia with a marked hemorrhagic component. The interstitial reaction with cells of the plasma-cell series points to the presence of immunomorphological changes, whereas the appearance of mast cells in the trachea and lungs reflects allergic changes.

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