DEVELOPMENT OF LUNG TUMORS IN SYRIAN HAMSTERS AFTER MIXED INFECTION WITH *Mycoplasma pneumoniae* AND INFLUENZA VIRUS

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Acute respiratory infections, including those of viral and microplasmal etiology, are the commonest of all diseases. Data in the literature on morphological changes in the lungs in experimental infection of animals with influenza virus are extremely abundant [4, 6, 7]. In this kind of infection marked circulatory disorders, dystrophic changes in the epithelium followed by its desquamation, and manifestations of regeneration have been described in the lung tissue. Several workers have drawn attention to metaplastic changes in the bronchiolar and alveolar epithelium and have suggested that in some cases these changes may lead to the development of neoplasms [5, 6, 12, 14]. There is also evidence that infection with small doses of influenza virus can lead to an increase in the percentage yield of tumors induced by chemical carcinogens in experimental animals [13, 15].

The features of mycoplasmal pneumonias have been described on the basis of a few morphological studies: perivascular and peribronchial lymphoid infiltration, in some cases infiltration resembling granulomas, consisting of histiocytes, an intrabronchial leukocytic exudate, vascular thrombosis, and circulatory disorders [1, 2, 10]. According to some workers [8] the distinguishing feature of mycoplasmal pneumonia is its transition into a chronic process, whereas according to others [2, 3], mycoplasmas can cause exacerbation of chronic pneumonia.

Most investigators have noted the important role of mixed infection with mycoplasmas and viruses in the etiology of acute respiratory diseases in children and adults. It has been shown [9] that combined mycoplasma-virus diseases constitute more than half of all recorded cases of pneumonia due to *M. pneumononiae*.

The aim of this investigation was to study morphological changes in the lungs of experimental animals after experimental mixed infection with a strain of *M. pneumoniae* and influenza virus.

EXPERIMENTAL METHOD

The test material consisted of the lungs of 18 male Syrian hamsters weighing 60-80 g, which were infected intratracheally, initially with a culture of *M. pneumoniae*† (titer 10^8-10^9 cfu/ml) in a dose of 0.1 ml per animal, followed 7 days later by influenza virus A/PR_8 in a dose of 10^3 in 0.05 ml. The comparison group consisted of 54 animals: 18 animals infected with a culture of *M. pneumoniae*, 18 animals receiving influenze virus, and 18 intact hamsters. The animals were killed under hexobarbital anesthesia 3 h, and 1, 7, 14, 21, and 28 days after infection.

Pieces of the lungs for light microscopy were fixed in Carnoy's fluid, and after treatment in the usual manner were embedded in paraffin wax. Sections 5-7 μ thick were stained with hematoxylin and eosin, with picrofuchsine by Van Gieson's method, by Brachet's reaction for RNA, and by the PAS reaction, with corresponding controls.

*Deceased.

†*M. pneumoniae* was isolated from a patient (strain 161/76).

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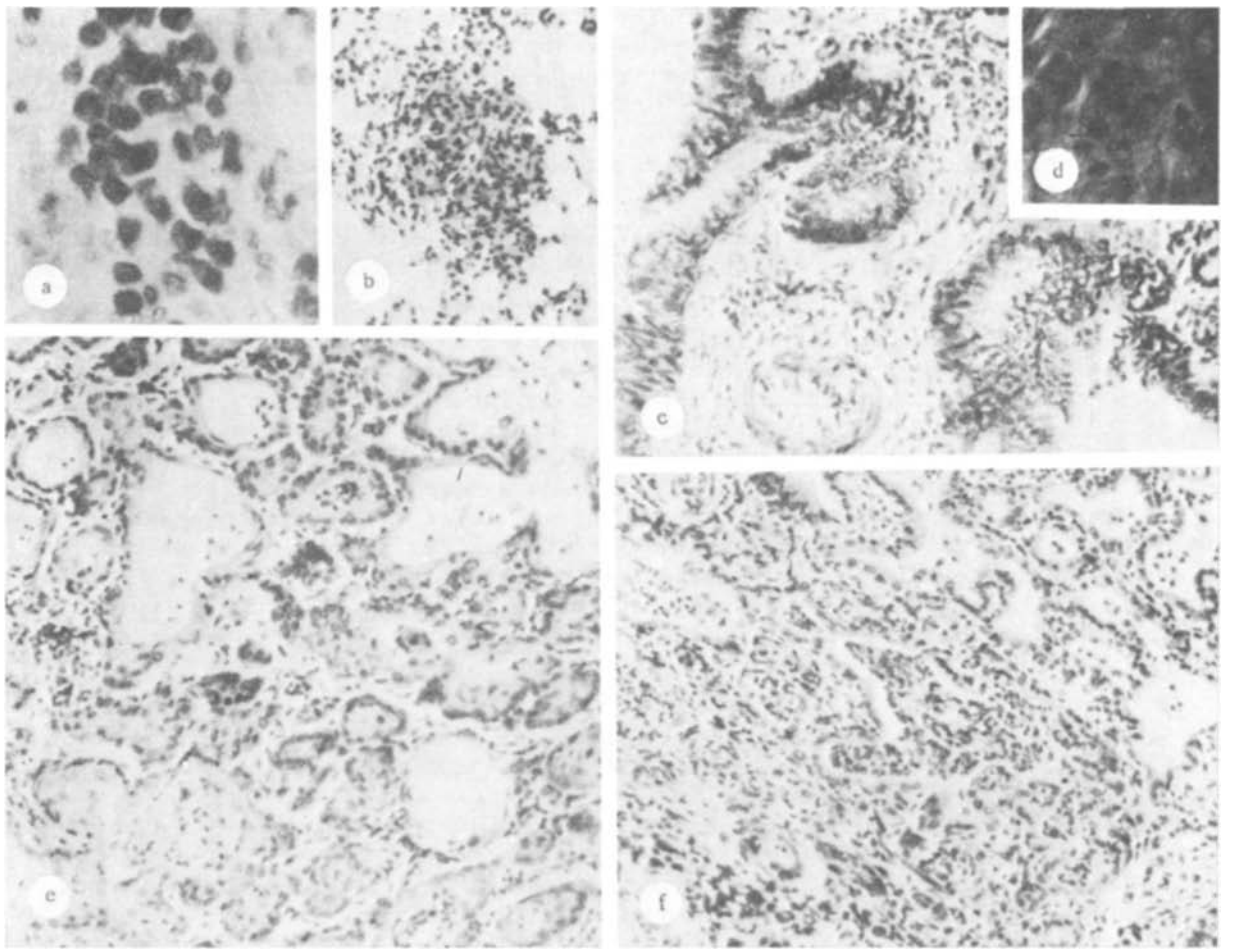

Fig. 1. Morphological changes in lungs of Syrian hamsters with mixed infection by *Mycoplasma pneumoniae* and influenza virus: a) perivascular focus of infiltration consisting of mature plasma cells (400 ×); b) focus of infiltration of "granuloma" type consisting of histiocytes (100 ×); c) proliferation of bronchiolar epithelium (100 ×); d) mitoses in cells of bronchiolar epithelium (400 ×); e, f) areas of lung tumors of varied histological strucrure: e) squamous-cell carcinoma (400 ×), f) carcinoma of glandular-solid type (100 ×). Stained with: a) methyl green and pyronine by Brachet's method; b-f) hematoxylin and eosin.

EXPERIMENTAL RESULTS

Morphological changes in the lungs of the Syrian hamsters with monoinfection corresponded to the pattern described in the literature [1, 2, 4, 5, 10]. The only point to note is that metaplasia of the bronchilar epithelium in infection with influenza virus was observed in 15% of the experimental animals. Metaplasia of the bronchiolar epithelium was not found in infection with *M. pneumoniae*. Marked circulatory disorders were found in the lungs 7 days after infection of the animals with *M. pneumoniae* and 3 h after additional infection with influenza virus: congestion of the vessels, hemorrhages, and a state of swelling of the endothelium and vessel walls. Changes observed in the bronchi were very varied: proliferation of epithelium, the formation of papilliform outgrowths, focal desquamation of the epithelium, and infiltration of the bronchial wall by leukocytes, and a leukocytic exudate in the lumen of the bronchi. There was a distinct response of the interstitial tissue of the lungs: Many foci of infiltration, mainly peribronchial and perivascular, and consisting of lymphocytes and large mature plasma cells (Fig. 1a), and also of infiltration of granuloma type, consisting of histiocytes (Fig. 1b), were found.

On the 1st-3rd days after infection with influenza virus all changes showed marked progression. Multiple foci of proliferation of the bronchiolar epithelium appeared in the lungs

(Fig. 1c). The epithelium appeared very juicy and swollen. Brachet's reaction revealed clear pyroninophilia of the epithelial cells, and many mitotically dividing cells were seen (Fig. 1d). At the later stages of the experiments the foci of proliferation were increased in size, and by the 14th-21st days after infection with influenza virus (21st-28th day of the experiment) the formation of tumors of mixed type, in which areas of squamous-cell carcinoma with "pearl" formation and carcinoma of glandular-solid type could be distinguished by their histological structure (Fig. 1e, f), formed in the lungs of the experimental animals.

In the group of animals with mixed infection metaplasia of the bronchiolar epithelium was observed in 93% of cases; in all four animals killed on the 21st-28th days of the experiment the development of lung tumors was noted.

It can accordingly be concluded from the results that infection with *M. pneumoniae* may facilitate the transition from metaplasis of the epithelium, which is frequently observed in influenza, into neoplastic growth. The mechanism of interaction between the infecting agents, and also of their combined effect on the host, requires elucidation. It will be evident that infection of Syrian hamsters with *M. pneumoniae* alone cannot induce tumor growth of the bronchiolar epithelium. Influenza virus is able to induce metaplasis of the bronchiolar epithelium. The most likely explanation is that additional tumor-generating information, transforming the cells, is introduced into the host by the influenza virus, and that infection with *M. pneumoniae* prepares the host for realization of the cytoproliferative action of influenza virus, exerting a varied influence on it, which may include certain kinds of action on the genetic apparatus of the cell.

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