Human Ciliated Epithelial Cells from Nasal Polyps as an Experimental Model for Mycoplasma pneumoniae Infection

MIRIAM ALMAGOR,1,2* ITZHAQ KAHANE,3 JOCHANAN M. WIESEL,3 AND SHAUL YATZIV2

Department of Membrane and Ultrastructure Research, The Hebrew University-Hadassah Medical School, Jerusalem 91010,1 and Department of Pediatrics2 and Laboratory of Experimental Surgery,3 Hadassah University Hospital, Jerusalem 91120, Israel

Received 16 October 1984/Accepted 1 February 1985

Mycoplasma pneumoniae is a specific human pathogen causing respiratory tract infections and various extrapulmonary complications (4, 6). During infection of the respiratory tract, M. pneumoniae adheres to the epithelial cells. Adherence is an important factor in the initiation of cell damage (6, 9).

The ability of M. pneumoniae to attach to a variety of human and animal cells in vitro (6, 7, 9–12) as well as to animal cells in vivo (8, 14) has been evaluated in previous studies. However, the majority of these studies were conducted with target cells that are not necessarily natural hosts for M. pneumoniae. Therefore, the development of an in vitro model system of human epithelial upper respiratory tract cells may be advantageous in studying various aspects of the relationship between M. pneumoniae and its host cells. In this work, cultured human ciliated epithelial cells derived from nasal polyps were used as an experimental system to study M. pneumoniae attachment and pathogenicity.

MATERIALS AND METHODS

Organisms and growth conditions. Virulent M. pneumoniae M129-B16 (obtained from J. B. Baseman, Health Science Center, University of Texas at San Antonio) were cultured as previously described (2) for 3 to 4 days at 37°C in Roux bottles containing modified Hayflick medium supplemented with 0.1 μCi of [3H]palmitate per ml. The cells were washed three times with sterile 0.25 M NaCl and were scraped off with a rubber policeman into sterile phosphate-buffered saline (PBS), pH 7.4. The suspended microcolonies were partially disaggregated by two to three transfers of the suspension through a 27-gauge hypodermic needle.

Cultured ciliated epithelial cells. Nasal polyps were obtained from patients undergoing nasal polypectomy due to nasal obstruction. The biopsy material was excised aseptically and washed with PBS containing 500 μg of penicillin and 500 μg of streptomycin per ml. Small explants were cut from the polyp surface, seeded on 35-mm tissue culture petri dishes (seven to nine pieces per dish), and covered with RPMI 1640 medium supplemented with 25% fetal bovine serum (GIBCO Diagnostics, Madison, Wis.). The cultures were incubated at 37°C under humidified air–5% CO2 as previously described (15).

Infection of cultured epithelial cells with M. pneumoniae. Cultures of ciliated epithelial cells were washed twice with sterile PBS, and the explant tissue pieces were removed. M. pneumoniae suspension in PBS (0.5 ml, containing 0.5 mg of protein) was added to each dish and incubated in nutrient mixture F-10 (GIBCO) supplemented with 3% fetal bovine serum, pH 7.4, at 37°C in 5% CO2 for up to 20 h. At the end of the incubation the cells were gently flushed twice with PBS to eliminate nonadherent mycoplasmas. For catalase activity, malondialdehyde (MDA) levels, and adherence assays, the cells were scraped off with a rubber policeman into PBS. The amount of radioactivity originating from the adherent mycoplasmas was assessed by scintillation spectrometry (2). The extent of nonspecific M. pneumoniae attachment to the plastic surface of the petri dishes was similarly evaluated.

Preparation for SEM. The samples were fixed with 2.5% glutaraldehyde in PBS, kept for 5 to 6 h at room temperature, and transferred to 4°C for 24 h. Dehydration was carried out with ethanol-Freon 113 as previously described (15). The samples were coated with a thin layer of gold-paladium and were examined in a JEOL 35 scanning electron microscope (SEM) equipped with an LaB6 gun at an accelerating voltage of 25 kV. Micrographs were recorded on Polaroid type 55 positive-negative film.

Catalase activity in intact cells. Cell catalase activity was measured as previously described (2) by following H2O2-dependent oxygen production at 30°C with an oxygen electrode (Yellow Springs Instrument Co., Yellow Springs, Ohio).

Lipid peroxidation assay. The extent of lipid peroxidation in cultured epithelial cells was estimated as MDA (a secondary breakdown product of fatty acids) by the thiobarbituric acid assay previously described (3). Protein was measured by the method of Lowry et al. (13).

* Corresponding author.
EXPERIMENTAL MODEL FOR M. PNEUMONIAE INFECTION

VOL. 48, 1985

- Images A, B, C, D, E, F show various stages of infection or interaction with M. Pneumoniae.
RESULTS

Morphology of cultured epithelial cells. The outgrowth area of the epithelial cells was clearly observed 3 to 4 days after seeding the explants, and rapid ciliary activity was evident on the monolayer of cells by microscopic examination (×100). The cell cultures were confluent within 14 days. The outgrowth monolayer was composed of two types of epithelial cells: one type had only stublike microvilli, and the other type had microvilli and cilia (Fig. 1A and B). The ciliated cells displayed long (6 to 8 μm), closely packed cilia (Fig. 1A, B, and C).

M. pneumoniae morphology and adherence to epithelial cells. M. pneumoniae microcolonies were spread over the epithelial cells; single filamentous organisms emerged from them in different directions and were oriented horizontally along the host cell membrane (Fig. 1D and E). The extent of adherence of M. pneumoniae to the host cells was calculated from the amount of radioactivity associated with the infected cells at the end of the incubation period. Attachment was observed within the first 15 min of incubation and increased linearly during the next 45 min (Fig. 2A). Longer periods of incubation had no significant effect on the degree of attachment (about 30% of the total counts). A linear increase in attachment was observed as a function of the number of mycoplasmas, at least up to 0.5 mg of cell protein (Fig. 2B).

Infected the epithelial cells with M. pneumoniae resulted in a decrease in local ciliary activity. The difference between infected and noninfected ciliated cells is shown in Fig. 1A, B, and F. M. pneumoniae cells were also found attached to the cell surfaces between the cilia (Fig. 1F).

Catalase activity and MDA levels. Catalase activity decreased to 25% of its normal value after 20 h of infection with M. pneumoniae, from 520 ± 140 to 130 ± 40 nmol of O₂ per mg of protein per min (mean ± standard deviation for four experiments). Since inhibition of intracellular catalase activity may result in oxidative damage to the infected cells due to increased intracellular H₂O₂ levels, the extent of lipid peroxidation was measured. The level of MDA in the infected cells, 2.52 ± 0.54 nmol/mg of protein, was 3.5 times higher than in controls (0.79 ± 0.16 nmol/mg of protein).

DISCUSSION

Natural infection with M. pneumoniae occurs only in humans (4, 7), the most common target cell being the ciliated respiratory epithelium. In the present study, human upper respiratory ciliated epithelial cells, derived from nasal polyps and cultured in a monolayer, served as an experimental model for studying host-parasite interaction at the cellular level.

The attachment of M. pneumoniae to the host cells was proportional to the number of mycoplasmas and seemed to follow first-order kinetics (Fig. 2). Similar results were reported for the attachment of M. pneumoniae to human erythrocytes (5, 12), WiDr cell culture, and hamster tracheal rings (5). The attachment of M. pneumoniae to the epithelial cells was followed by disorganization and decrease in ciliary activity as observed by light microscopy. The ciliostatic effect of M. pneumoniae infection has also been shown in infected hamster tracheal organ cultures (11). In our study the observed morphological changes were accompanied by a marked decrease in intracellular catalase activity, followed
by a significant increase in MDA levels in the infected cells. The observed rise in MDA levels may have resulted from the increased intracellular levels of H$_2$O$_2$ caused by host cell catalase inhibition. The results presented, in accordance with our previous studies with *M. pneumoniae*-infected human cells (1, 2), indicate that *M. pneumoniae* infection induces oxidative damage to the host cells. The data presented here suggest, therefore, that human nasal ciliated epithelial cells are applicable as an experimental model for studying the relationship between *M. pneumoniae* and its host cell. Ciliated epithelial cells, as opposed to human fetal tracheal organ cultures (7), are more easily available in large amounts and can be maintained in a routine cell culture laboratory. Furthermore, as these cells grow in a monolayer, intracellular studies can be performed without the interference of other types of cells naturally present in animal (10) and human tracheal organ cultures.

The ciliated epithelial cells derived from nasal polyps originate in the respiratory tract and possess morphological characteristics similar to those of other cells in this system (15). We suggest that these cells are representative of the in vivo host cells and can therefore serve as a reliable model for studying various processes associated with *M. pneumoniae* attachment and pathogenicity.

LITERATURE CITED