Effect of Chlamydia trachomatis Infection on Ciliary Activity in Single Cells from Cultures of Human Nasal Polyps

By ESTHER LUZZATTO, GIDEON KOPERNIK, ISRAEL SAROV* AND ZVI PRIEL

Department of Chemistry, Faculty of Natural Sciences, and Virology Unit, Division of Obstetrics and Gynecology, Faculty of Health Sciences and Soroka Medical Center, Ben Gurion University of the Negev, Beer Sheva 84105, Israel

(Received 3 May 1988; revised 12 August 1988; accepted 29 September 1988)

We have tested the hypothesis that the ciliary activity of epithelial cells from human nasal polyps is altered after infection with Chlamydia trachomatis. Ciliated epithelial cells from human nasal polyps were cultured and infected with C. trachomatis. The measurement of ciliary beating was based on a technique which enables one to monitor a fraction of a single ciliated cell. A marked decrease of ciliary beating frequency versus time was observed 24 h after infection with C. trachomatis. About 50% of the cilia of infected cells were paralysed 48 h post-infection. The potential effect of C. trachomatis infection on the physiological functions dependent on cilia is discussed.

INTRODUCTION

Chlamydia trachomatis is the causative agent of trachoma, the major reason for human blindness in developing countries. In addition, this organism has been recognized recently as a common cause of a variety of sexually transmitted diseases such as non-gonococcal urethritis, epididymitis, cervicitis, endometritis and salpingitis. Also it causes obstructive infertility and respiratory tract diseases such as pneumonitis (Ladany & Sarov, 1985). C. trachomatis is an energy parasite (Weiss, 1965) that consumes ATP produced in the host cell (Hatch, 1982); it might thus be expected to have a negative effect on ciliary activity, which is dependent on ATP supplied by the same cell. However, studies by Phillips et al. (1984) and Hutchinson et al. (1979), in murine and bovine oviducts, respectively, in which ciliary motion was monitored by light microscopy, indicated no reduction in ciliary activity after infection by C. trachomatis.

The aim of the present work was to re-examine the hypothesis that C. trachomatis may influence cilia, especially ciliary beating. This was done by studying ciliated cells from cultures of human nasal polyps.

METHODS

Cell cultures. The method of preparation of human nasal polyp cultures was modified from that described by Wiesel et al. (1983). Human nasal polyps were obtained from 20 patients in the range 5 to 40 years of age (mean 10 years). The polyps were placed in PBS solution (2.6 mM-KCl, 8 mM-Na2HPO4, 1.4 mM-KH2PO4, 135 mM-NaCl, pH 7.2–7.3) containing 500 µg streptomycin ml–1 and 500 µg penicillin ml–1 and were washed three times. The ciliary layer was peeled off under a binocular microscope and cut into 1 mm squares. Each piece was seeded on a 3.5 cm plastic Petri dish (Nunc) which had been pre-incubated for 1 h in a CO2 incubator with 1 ml RPMI-1640 growth medium containing 10% (v/v) foetal calf serum (FCS), 2.1 mM-glutamine, 0.55 mM-glucose, 100 µg streptomycin ml–1, 10 µg gentamicin ml–1 and 10 µg fungizone ml–1 (Biolab, Jerusalem).

After 4 d, an outgrowth of active ciliated epithelial cells and non-ciliated cells began to appear around the explant. The medium was carefully replaced every 2–3 d. The ciliary activity remained stable for about three weeks.

Abbreviations: EB, elementary body; RB, reticulate body; FCS, foetal calf serum; IFU, inclusion-forming units.
Preparation of infectious elementary bodies (EBs). C. trachomatis biovar lymphogranuloma venereum (L2/434/Bu) was grown in MA-104 cells from embryonic Rhesus monkey kidney (Microbiological Associates), in RPMI-1640 growth medium supplemented with 5% (v/v) FCS, 0.55 mM-glucose, 0.178 mM-sodium bicarbonate, 2 mM-L-glutamine, 100 µg streptomycin ml⁻¹, 10 µg fungizone ml⁻¹ and 1 µg cycloheximide ml⁻¹. Chlamydia were harvested from MA-104 monolayers grown in polystyrene flasks (basal area 175 cm²; Nunc) as described by Caldwell et al. (1981). Purified EBs were suspended in SPG buffer (0.01 M-sodium phosphate, pH 7.2, containing 0.25 M-sucrose and 5 mM-L-glutamic acid) and stored at −70 °C until used.

Immunoperoxidase assay for titration of C. trachomatis. C. trachomatis was titrated on MA-104 cells as described previously (Shemer & Sarov, 1985). The cells were seeded at 2 × 10⁴ to 3 × 10⁴ cells per well in 96-well microtitre plates (Nunc). After 48 h, triplicate 50 µl samples of serial 10-fold dilutions of chlamydial inoculum in growth medium were added. The cells were fixed 2 d later with 100% ethanol, and an immunoperoxidase assay modified for chlamydia (Shemer & Sarov, 1985) was performed. The final results of titration were expressed as inclusion-forming units (IFU) ml⁻¹.

Infection of ciliated cells with C. trachomatis. About 6 d after seeding, the cultures were washed with minimal essential medium (MEM) (Biolab, Jerusalem) containing 5% (v/v) FCS, 0.55 mM-glucose, 0.178 mM-sodium bicarbonate, 100 µg streptomycin ml⁻¹, 10 µg gentamicin ml⁻¹ and 10 µg fungizone ml⁻¹. After 24 h in this medium, the cultures were washed with MEM medium plus 1 µg cycloheximide ml⁻¹. Purified C. trachomatis was added to the medium at a final concentration of 4 × 10⁵ IFU ml⁻¹. The infected tissue cultures were kept in a CO₂ incubator at 37 °C.

Measurements of ciliary beating frequency. Measurements of ciliary beating frequency were made with an inverted light microscope with MIT-13 phase-contrast optics (Olympus). Light was supplied by a 100 W tungsten lamp which was powered by a home-made DC supplier (Eshel & Myers, 1976). One ocular was replaced by a 700-10-36 A ocular (Gamma Scientific) with a fixed optical fibre, positioned in its optical centre. The diameter of the area of observation was 2.5 µm as defined by the diameter of the fibre (50 µm) divided by the objective magnification (× 20). An additional elastic optical fibre (700-3C, Gamma Scientific) was connected at one end to the fixed fibre and at its other end to a photomultiplier (EMI). High voltage was supplied to the photomultiplier by a 451B source (Fluke). The amplified analogue signals were digitized and stored by an Apple IIe micro-computer with an A/D converter (Mountain) with an analogue range of −5 to +5 V, a resolution of 8 bits and 16 input channels. A sampling rate of 360 Hz was employed. The data acquisition system and the computer analysis were calibrated with a 10 Hz oscillating light, with intensity matched to that obtained from the tested tissue (Eshel et al., 1985). The main advantages of this computerized electro-optical system are: (i) it permits measurement of relatively small areas (a fraction of one cell) with high accuracy (the signal-to-noise ratio is higher than 10); and (ii) the computerization of the system, makes it relatively easy to store and analyse the signals obtained. Fig. 1(a) shows a typical signal taken from ciliary epithelium. Fig. 1(b) shows the noise of the system taken under the same conditions from a non-ciliated area, while Fig. 1(c) shows the power spectrum of the signal.

The time-dependent signal obtained through light changes detected by the optical fibre, as a result of the ciliary beating, was analysed by fast Fourier transform (FFT) to obtain spectra (Fig. 1(c)) which very accurately define the main frequencies of ciliary beating. The spectra contained information on the frequencies sampled during 1 s, and 47 such 1 s spectra were obtained in each case. From each 1 s spectrum, the frequency presenting the highest intensity was found, as well as the standard deviation of such frequencies. It was shown by Eshel & Priel (1986) that under such conditions the optical signal is optimal, and a well-defined ciliary beating frequency can be derived. Each run was carried out during 7 d and all tissues were measured twice a day. About 112 measurements were made for each data point, and the values of the frequency obtained were correct with a confidence interval of 99% (Walpole & Myers, 1976).

RESULTS

Tissue culture of human nasal polyps

Outgrowth of the cultures was observed after 4 d, with some of the cells showing ciliary activity. Typical inclusions could be seen by phase-contrast microscopy, in ciliated and non-ciliated cells, 24 h after infection of cultures with C. trachomatis. At this stage, five clearly distinguished infected and non-infected ciliated cells were marked with circles beneath each Petri dish, and were examined for ciliary activity. This procedure was repeated on ten different sets of samples, comprising altogether 100 measured cells, half of which were infected and the other half control.

Both ciliated and non-ciliated cells showed large inclusion bodies 48 h after infection. Many EBs and reticulate bodies (RBs) were seen after Giemsa staining. About 72 h post-infection, bursting of the vacuoles was also evident.
Fig. 1. A typical signal and spectrum of ciliary beating frequency measured by the computerized electro-optical system. (a) Analogue output of optical signal from the ciliated area examined (field diameter 2.5 μm). (b) Reference signals recorded from an area of non-ciliary epithelium. (c) One-second Fourier-transformed spectrum. The spectrum was normalized with respect to its highest power. \( s(f) \) is power density and \( s(f)_{\text{max}} \) is the maximum power density of the spectrum.

Fig. 2. Results of the normalized ciliary beating frequency versus time post-infection in (a) non-infected and (b) infected cells. Normalization to the mean frequency of each experiment at \( t = 0 \). Ten different experiments are plotted. Each experiment at any given time is an average of 560 (112 spectrum measurements × 5 different cells) 1s spectra.
Effect of C. trachomatis on the ciliary beating frequency

Typical plots of the normalized frequency versus time in infected and non-infected ciliated cells are illustrated in Fig. 2. The change in frequency with time in infected cells is composed of two stages: (i) no measurable change in the ciliary beating frequency at 24 h post-infection, and (ii) a subsequent marked decrease in frequency as a function of time post-infection. About 50% of the cilia of the infected cells were paralysed at 48 h post-infection. Cilia which were not paralysed showed a decrease of about 40% in beating frequency after 48 h, and had ceased to beat at 96 h post-infection.

Although no attempt was made to measure the number of cilia per cell, it was clear by visual inspection that the density of cilia per cell markedly decreased after infection.

DISCUSSION

The hypothesis that C. trachomatis infection of ciliated cells from human nasal polyps affects ciliary activity was confirmed. According to Hatch (1982), in the first stage of infection only the EB (the infective form) is present within the cell, and C. trachomatis does not consume ATP while undergoing conversion to the RB. This stage lasts about 24 h and is followed by a requirement for ATP. This might explain the results of the present study in which no effect on ciliary beating was observed during the first 24 h post-infection. The observed inhibition of ciliary activity after 24 h may be attributable to competition for ATP between the chlamydial and ciliated cell, or may result from an effect on cellular function not directly related to ATP competition. Further studies are required to elucidate the mechanism by which C. trachomatis infection inhibits ciliary activity.

Inspection by light microscopy revealed a decrease in the number of cilia on infected cells. Donnez et al. (1984) reported a considerable decrease in the number of cilia in the fimbriae of human oviducts of patients suffering from salpingitis. The mechanical infertility observed in these women may be a manifestation of this decrease. The present results contrast with those of Phillips et al. (1984) and Hutchinson et al. (1979), who found no effect of chlamydia on ciliary activity. This apparent inconsistency is probably due to the following. (i) In previous studies the measurements were made on relatively large samples containing both infected and non-infected cells. Examination of the whole tissue can mask the effect under study as a result of contributions to the measurement by the non-infected cells which continue to beat normally. In the present work, the accuracy of the measurements was considerably increased by monitoring single infected cells as a function of time. (ii) The above authors evaluated the ciliary activity on the basis of direct visual inspection, as described by MacGee et al. (1976). This method can provide only a rough evaluation of ciliary activity. We have found that changes of ±3 Hz may be equivalent to a decrease of about 50% in beating frequency. Such changes can not be detected by visual inspection of ciliary movement because of intrinsic fluctuation in ciliary beating. A relatively accurate measuring apparatus is needed to detect such changes and even then relatively big ensembles of measured data are needed in order to get a reliable average. Our system enables the detection of such small changes and the creation of big ensembles so that the frequency of ciliary beating may be measured with high accuracy (see Methods).

In conclusion, the present study demonstrates that infection by C. trachomatis (L2/434/Bu) causes a decrease of ciliary activity in cultured human nasal polyp cells. Future investigations should consider the effects of other chlamydial serovars on cultures of appropriate ciliated cells. The sexually transmitted serovars D–K, associated with salpingitis, mechanical infertility and pneumonitis are of particular interest. It may be that a decrease in ciliary activity in the early stages of C. trachomatis infection is an important factor contributing to infertility (Punnonen et al., 1979). Similarly, studies using C. psittaci, and the TWAR organism (Marrie et al., 1987) associated with respiratory illness and pneumonia, could provide insight into the contribution of reduced ciliary activity to the pathogenesis of respiratory infections.
REFERENCES


