Further characterization of an outer membrane protein of *Chlamydia trachomatis* with cytadherence properties

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1. SUMMARY

To further characterize the chlamydial cytadhesin (CCA), we have examined it for saturability of binding to HeLa cells that were grown as monolayers and in suspension. The CCA exhibited specific cytadherence properties of binding to HeLa cells that appeared to be saturable. The CCA showed a substantial decrease in binding to trypsin-treated HeLa cells in suspension. This finding, together with the fact that the CCA itself is known to be trypsin-sensitive, suggested a protein–protein type of interaction between CCA and HeLa cells. Periodate treatment of the CCA did not result in significant reduction in cytadherence, which implies that sugar moieties were probably not involved in CCA binding to HeLa cells. Whilst attempts to produce antibodies to the CCA in rabbits was unsuccessful, the CCA reacted with antibodies in a human serum known to contain high titer antibodies to *Chlamydia trachomatis*, suggesting it can be immunogenic, and is possibly expressed during human infection.

2. INTRODUCTION

*Chlamydia trachomatis* is an obligate intracellular bacterial pathogen responsible for a variety of diseases in humans. Similar to other intracellular parasites, this bacterium must attach to susceptible host cells before entering the cell and initiating replication. Presumably this attachment occurs through the interactions of a chlamydial surface protein(s) and specific cell surface receptors. The kinetic properties of chlamydial attachment to host cells has been studied in detail, but little is known of the surface components of the bacterium mediating specific adherence [1]. In a recent communication [2] we reported the identification of a chlamydial cytadhesin (CCA). This CCA is extracted from highly purified elementary bodies (EB), with the non-ionic detergent, *n*-octyl-*β*-d-glucopyranoside (OGP), and binds to the surface of HeLa cells. CCA binding to the HeLa...
cells is specific, as shown by the fact that viable EB compete with the CCA for binding to the host cell. In addition, preincubation of the HeLa cells with the CCA-containing extract results in significant reduction in the adherence of labelled EB and the development of chlamydial inclusions. The CCA is a minor component of the EB outer membrane and available only in small quantities in the OGP extract of EB. This paucity of the CCA and the fact that tissue culture propagation of Chlamydia and subsequent EB purification steps are time-consuming, limit elaborate studies on the CCA. This investigation extends our earlier study on the binding of CCA to HeLa cells and further examines its cytadherence properties.

3. MATERIALS AND METHODS

3.1. Preparation of an extract containing CCA

C. trachomatis serovar E was grown in HeLa cells, the EB were purified and the surface-exposed proteins were labelled extrinsically with $^{125}$I [2]. The labelled EB was resuspended in phosphate-buffered saline (PBS) containing 2% (w/v) OGP and 1 mM phenylmethyl-sulfonyl fluoride, and a soluble extract, the OGPE, was obtained.

3.2. Cytadherence assay of chlamydial protein in the OGPE

The gel electrophoresis-autoradiography assay to detect cell-bound $^{125}$I-labelled chlamydial protein in the dialysed OGPE using glutaraldehyde-fixed HeLa cell monolayers in 96-well culture dishes has been described [2].

3.3. Cytadherence of the CCA to HeLa cells in suspension

HeLa 229 cells grown as monolayers were detached by incubation with PBS containing 10 mM EDTA. The cells were washed thrice with PBS and then resuspended in 2.5% glutaraldehyde in PBS. After 1 h at 4°C, the cells were washed extensively with PBS and resuspended in PBS containing 2% (w/v) bovine serum albumin. After overnight storage of the cells at 4°C, the cells were washed thrice with PBS and resuspended at $2.9 \times 10^6$ ml$^{-1}$. For each assay, 0.5-ml aliquots of cells were incubated with different inputs of the dialysed $^{125}$I-labelled OGPE (0.25 μg protein μl$^{-1}$; 39–66 × 10$^3$ cpm μl$^{-1}$) for 1 h at 4°C in microcentrifuge tubes. This reaction was terminated by the addition of 1 ml of PBS, and the cells were pelleted by a brief centrifugation. After washing extensively with PBS, the cells were transferred to clean tubes and re-centrifuged. The pellets were solubilized [3] and subjected to electrophoresis (SDS-PAGE) in 12.5% gels followed by autoradiography.

Monolayer cultures of HeLa cells were detached with trypsin (100 μg per ml) in the presence of cycloheximide [4] (10 μg ml$^{-1}$), washed extensively with PBS and then fixed with glutaraldehyde as described above.

3.4. Treatment with metaperiodate

The OGPE from $^{125}$I-labelled EB was incubated with freshly prepared 2 mM sodium metaperiodate in PBS for 30 min at 0°C in the dark, and the reaction was quenched with 0.1 volume of 0.1 M glycerol in PBS [5]. The cytadherence activity of the untreated and the periodate-treated extracts was assayed.

3.5. Immunoblotting

For the immunoblotting assays, the OGPE containing the CCA was concentrated by ultrafiltration. The concentrated OGPE and the lysate of the EB were subjected to 12.5% SDS-PAGE. To obtain a well-separated band of the CCA, the 12.5% gel was run at 100 V until the dye front entered the separating gel; the gel was then run at a constant current of 15 mA for 17 h. The buffer was replaced and the gel was run for an additional 3 h at 35 mA and the proteins were electroblotted overnight using 10 mM 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS) (Sigma), pH 11, and 10% methanol [6]. The PVDF membrane (Immobilon P, Millipore) was blocked with 5% non-fat dry milk in PBS for 30 min and then incubated overnight with a 1:100 dilution of a human serum [7]. The membrane was washed with 0.025% Tween-20 in PBS and incubated with a 1:500 dilution of goat-anti-human immunoglobulins conjugated to horseradish peroxi-
dase (Tago). Color development was with 4-chloro-1-naphthol.

4. RESULTS AND DISCUSSION

*C. trachomatis* serovars B, E, and L1 that are grown either in HeLa or McCoy cells exhibit CCA of identical $M_r$ [2]. The SDS-PAGE electrophoresis of the OGPE of *C. trachomatis* serovar E, used in the present study, was carried out for longer time periods resulting in a better separation of the protein bands and a more accurate estimation of the $M_r$ of the CCA. Previously we had estimated the $M_r$ to be 38 [2], but the present study indicated that the CCA co-migrated with the glyceraldehyde-3-phosphate dehydrogenase marker (Sigma) at $M_r$ 36 (Fig. 4). When increasing amounts of the dialysed OGPE were incubated with glutaraldehyde-fixed monolayers of HeLa cells, a dose-dependent increased binding of the CCA was observed after SDS-PAGE and autoradiography of the samples (Fig. 1A, lanes 2–7). A representative autoradiograph was analysed by densitometry and absorbance values of the cell-bound CCA were plotted as a function of OGPE input. With increasing amounts of the OGPE, the amount of the $^{125}$I-labelled CCA that bound to the host cells appeared to reach saturation (Fig. 1B), indicating a dose-dependent and specific interaction of the CCA with the receptors on the host cell surface. As had been noted before [2], while other iodinated proteins present in the OGPE also bind to the host cells with high inputs of the OGPE (Fig. 1A, lanes 6 and 7), densitometry of the autoradiographs revealed a lack of saturable binding of these proteins (data not shown). The binding of these other OGPE proteins to HeLa cells is non-specific as they are also known to bind the cell-free plastic surface of the culture dish [2].

For determining the effect of trypsin treatment of HeLa cells to the binding of the CCA, the monolayer was detached with trypsin (100 µg per ml) in the presence of 10 µg per ml cycloheximide to prevent the rapid regeneration of the chlamydial receptor [4]. Untreated and trypsin-treated HeLa cells in suspension were incubated with OGPE containing $^{125}$I-labelled CCA. Cell-bound iodinated chlamydial proteins were detected after SDS-PAGE and autoradiography. Figure 2 is a representative experiment showing

![Figure 1](image-url)

**Fig. 1.** A. Dose-dependent cytadherence of the CCA to glutaraldehyde-fixed HeLa cell monolayers in a 96-well dish. Increasing amounts of the OGPE containing $^{125}$I-labelled chlamydial proteins were incubated with fixed HeLa cells at 4 °C for 1 h. After washing, the cells from duplicate wells were solubilized, and the cell-bound labelled proteins were detected by SDS-PAGE followed by autoradiography. Arrow shows the position of the CCA in the OGPE (lane 1). The amounts of protein in the OGPE per well were: lane 2, 0.625 µg; lane 3, 1.25 µg; lane 4, 2.5 µg; and lane 5, 3.75 µg; lane 6, 5.0 µg; and lane 7, 6.25 µg. Molecular mass markers are shown on the left. B. Saturation of CCA binding to fixed HeLa monolayers quantitated by densitometry of the autoradiograph shown in Fig. 1A. Densitometry readings could not be obtained for lane 7 shown in Fig. 1A, because of the dark background. However, visual observation showed no increase in intensity of the CCA band in lane 7 with a higher input of OGPE.
Fig. 2. Binding of CCA to untreated and trypsin-treated HeLa cells in suspension. Details are provided in MATERIALS AND METHODS. Lane 1, the OGPE; lane 2, 125I-labelled protein bound to HeLa cells (2.5 μg protein in the OGPE); and lane 3, trypsin-treated cells incubated with the same input of OGPE. Arrow points to the CCA. Note the substantial decrease (64%) of binding of the CCA to trypsin-treated HeLa cells. Molecular mass markers are shown on the left.

Fig. 3. Cytadherence activity of the CCA after periodate treatment. Lane 1, the OGPE; lane 2, 125I-labelled protein bound to HeLa cells (1.25 μg protein in the OGPE); and lane 3, periodate treated 125I-labelled protein bound to HeLa cells when incubated with the same input of OGPE. Densitometric scan of the CCA band in lane 3 showed a 28% reduction of binding to HeLa cells after periodate treatment. Arrow points to the CCA. Molecular mass markers are shown on the left.

Fig. 4. Reactivity of high titer human serum with the CCA. Lanes 1 and 2, autoradiograph of 125I-labelled OGPE, and HeLa cell-bound CCA; lanes 3 and 4, immunoblot of whole EB lysate and OGPE reacting with the human serum as described in MATERIALS AND METHODS. Arrow points to the CCA. The positions of the molecular mass markers including the 36-kDa glyceraldehyde-3-phosphate dehydrogenase are shown on the left.
reported by Kaul et al. [8] based on trypsin sensitivity of HeLa cells to EB binding. Their data thus corroborate our observation on the protein nature of the interaction between CCA and HeLa cells. We looked further into the CCA–HeLa cell interaction by measuring cytadherence activity of the CCA after periodate treatment. Figure 3 is a representative experiment showing cytadherence activity of CCA after periodate treatment. Densitometry of the autoradiograph shown in Fig. 3 demonstrated a decrease in binding of only 28%, implying that sugar moieties are probably not involved in the CCA binding to HeLa cells.

We attempted to produce polyclonal anti-CCA antibodies for immunoaffinity purification of the CCA. Rabbits were injected with macerated SDS-PAGE gel slices containing the CCA and Freund's complete adjuvant. No anti-CCA activity could be detected in the sera after repeated boosters. However, a human serum (case 3) [7] containing a high titer of antibodies to *Clamydia* reacted with the CCA in immunoblot assays. This reactivity is shown in the immunoblot displayed in Fig. 4, where reactivity was observed with the CCA in whole EB lysate (lane 3) as well as in the concentrated OGPE (lane 4). Preliminary immunoblot data using this human serum and HeLa cell-bound OGPE, demonstrated the selective binding of only the CCA in the chlamydial extract. Taken together, these findings suggest that the CCA can be immunogenic and its proper presentation to the host animal may be dependent on its conformation. The presence of antibodies to CCA in serum from infected human patients suggests that CCA is expressed in vivo during infection.

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