SHORT COMMUNICATION

Antibody-neutralizing activity against all urogenital *Chlamydia trachomatis* serovars in *Chlamydia suis*-infected pigs

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Abstract

It is known that neutralizing species-specific or serovar-specific antibodies are produced in response to chlamydial infection in humans and in some animal species. In a previous study, a strong *in vitro* neutralizing activity to *Chlamydia suis* in 80% of sera from *C. suis*-infected pigs had been observed. In view of the close relationship between *C. suis* and *Chlamydia trachomatis*, in the present study, the neutralizing activity against D–K *C. trachomatis* and *C. suis* purified elementary bodies (EBs) in sera collected from *C. trachomatis*-infected patients and *C. suis*-infected pigs was evaluated. A neutralizing activity of 50–70% was observed in the human sera against the homologous serovar and one to five heterologous *C. trachomatis* serovars. These sera were also able to neutralize *C. suis* EBs. The pig sera showed a strong neutralizing activity (70–100%) against *C. suis* EBs and all eight urogenital *C. trachomatis* serovars. These results suggested the presence of common immunogenic antigens in *C. trachomatis* and *C. suis*. Immunoblot analysis, performed to elucidate the target of this neutralizing activity, showed a clear reactivity in human and pig sera against two proteins of 150 and 40 kDa MW, when tested either with *C. trachomatis* or with *C. suis* EBs.

It is known that neutralizing species-specific or serovar-specific antibodies are produced in response to chlamydial infection in humans and in some animal species (Banks et al., 1970; Peterson et al., 1990; Girjes et al., 1993; Donati et al., 1996, 2006, 2009). The detection of these antibodies could be useful in the diagnosis of mixed infections or in the detection of immunogenic antigens as vaccine candidates.

A previous study (Donati et al., 2009) reported a strong *in vitro* neutralizing activity to *Chlamydia suis* in 80% of pig sera that, due to the presence of high microimmunofluorescence (MIF) titres, suggested *C. suis* infection. A close relationship between *C. suis* and *Chlamydia trachomatis* has already been reported in relation to the ompA DNA sequence similarity (Kaltenboeck et al., 1997), together with morphology and other features, such as the production of glycogen in cell culture (Rogers et al., 1996) and the sensitivity to cathelicidins (Donati et al., 2007).

In view of these features, in the present study, we evaluated the neutralizing activity against D–K *C. trachoma-
Table 1. Neutralizing activity of sera from *Chlamydia trachomatis*-infected patients and *Chlamydia suis*-infected pigs against the eight *C. trachomatis* serovars and *C. suis* 7MS06 EBs

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*Chlamydophila psittaci* 6BC and the Italian *Chlamydophila felis* FEIS-M isolate were used to check the species-specificity of neutralizing antibodies in the human and pig sera.

EB preparations were titrated to contain $4 \times 10^3$ inclusion-forming units (IFU) mL$^{-1}$ and stored frozen in 0.25 M sucrose–10 mM potassium phosphate–5 mM glutamic acid, pH 7.4 (SPG), at $-70^\circ$C. As a source of complement, aliquots of fresh rabbit serum were stored at $-70^\circ$C and used in the neutralization assay at a 5% final concentration. Human and pig sera were heat-inactivated at 56$^\circ$C for 30 min, diluted 1 : 5 in Hanks balanced salt solution (HBSS) and distributed (100 $\mu$L) in triplicate over 96-well microtitre plates. EB stock was then diluted in SPG to contain $2 \times 10^4$ IFU mL$^{-1}$, and 90 $\mu$L was added to prediluted sera, and to HBSS (100 $\mu$L) for control. The serum–EB mixtures, incubated for 30 min at 37$^\circ$C, were then inoculated in triplicate into LLC-MK2 cells grown in 24-well plates, including a glass coverslip at the bottom, and chlamydial growth medium (800 $\mu$L) was added, thus obtaining a final serum dilution of 1 : 10. After a centrifugation at 1000 $g$ for 1 h, the monolayers were incubated at 37$^\circ$C for 48 h and then fixed in methanol and stained with a fluorescein-conjugated monoclonal antibody specific for the chlamydial lipopolysaccharide genus-specific antigen. Ten fields/well (at a magnification of $\times 200$) were read through the midline of the coverslip, in the test and control assays. An average was taken and the results were expressed as percent reduction of IFU from control monolayers.

All determinations were performed at least twice on different days. A $\geq 50\%$ reduction from control IFU in infectivity was defined as neutralization.

The sera that were positive at the final dilution of 1 : 10 were tested again at dilutions of 1 : 20 and 1 : 40 in the presence/absence of complement, to determine the neutralizing titre.

Human sera neutralized the homologous serovar and 1–5 heterologous serovars of *C. trachomatis*. The mean neutralizing activity against the homologous and heterologous serovars was 80% and 60%, respectively. These sera were also able to neutralize *C. suis* EBs, with a mean neutralization activity of 68%. All pig sera strongly neutralized *C. suis* EBs and all eight serovars of *C. trachomatis*, showing a mean neutralizing activity of 100% and 91%, respectively (Table 1, Fig. 1). Sera showing a neutralizing activity of 90–100%, when diluted 1 : 10, were able to neutralize at the dilution of 1 : 20–1 : 40 in the presence of complement and of 1 : 10–1 : 20 in the absence of complement, whereas sera with a neutralizing activity < 90% at the dilution of 1 : 10 resulted neutralizing at the dilution of 1 : 10–1 : 20 in the presence of complement and at the dilution of 1 : 10 or not neutralizing in the absence of complement.

Neither human nor pig sera were able to neutralize *C. muridarum*, *C. pneumoniae*, *C. psittaci* and *C. felis* EBs.

Control sera showed no neutralizing activity against the chlamydial species tested.

An immunoblot analysis was performed to elucidate the target of this neutralizing heterospecific activity. Italian *C. trachomatis* isolate D and *C. suis* 7MS06 purified EBs were treated with a solubilizing solution and boiled for 10 min as described by Caldwell et al. (1981). The polypeptides were separated by sodium dodecyl sulphate-polyacrylamide gel
electrophoresis (SDS-PAGE) (Laemmli, 1970), using a 12% (w/v) precast gel (Invitrogen). The Recombinant Molecular Weight Marker (Sigma) was used as a molecular mass marker. Electrophoresis was carried out in a vertical slab gel apparatus (Bio-Rad, Hercules, CA) at a constant current using 30 mA for 1 h. Subsequently, the separated polypeptides were electrotransferred for 1 h to nitrocellulose paper (Sigma) using a mini transblot cell (Bio-Rad). The nitrocellulose paper, stained with Ponceau-S (0.1% in 1% acetic acid) to ensure the transfer of proteins, was then cut into strips. The strips were blocked with 5% albumin in phosphate-buffered saline (PBS) for 1 h at room temperature and washed three times in PBS, pH 7.4, containing 0.05% (v/v) Tween 20 (PBST). Subsequently, the strips were incubated with sera diluted 1 : 100 in PBST, under gentle agitation. After washing the strips three times by PBST, antigen–antibody complexes were detected by incubating the strips for 2 h at room temperature with peroxidase-labelled goat anti-human IgG (Dako, Glostrup, Denmark) diluted 1 : 500 in PBST or anti-swine IgG (KPL, Kirkegaard and Perry Laboratories, Gaithersburg, MD) diluted 1 : 2500 in PBST, and using 4-chloro-naphthol (Bio-Rad) as the enzyme substrate.

Both human and pig sera showed a clear reactivity against two proteins of 150 and 40 kDa MW, when tested either with *C. trachomatis* or with *C. suis* EBs (Fig. 2).

As regards the results of our study, the neutralizing activity of each human serum against at least two serovars of *C. trachomatis* could be due to a cross-reacting serovar or previous infections with different serovars. More interesting are the data on the neutralizing activity of pig sera against all the eight *C. trachomatis* serovars tested, suggesting the presence of common immunogenic antigens able to generate heterospecific and heterotypic neutralizing antibodies.

With regard to the immunoreactivity against the 40 kDa (MOMP) protein, several studies have focused on this protein as a possible vaccine candidate, because it is highly immunogenic, immunoaccessible and a target of neutralizing antibodies. However, the protective MOMP-related immunity has been shown to be serovar specific, with little to no cross-protection against different serovars (Dawson *et al.*, 1967; Tarizzo *et al.*, 1967; Grayston *et al.*, 1971; Taylor, 1990; Kari *et al.*, 2009).

Recently, Crane *et al.* (2006) showed that all *C. trachomatis* reference serotypes synthesize a 155 kDa highly conserved surface-exposed antigen termed polymorphic membrane protein D, generating neutralizing antibodies against all *C. trachomatis* serovars, but that failed to neutralize *C. muridarum*. At present, no studies have been performed on polymorphic membrane proteins in *C. suis*. The close biological relationship between *C. suis* and *C. trachomatis* could suggest a strong similarity between the polymorphic membrane proteins of these two chlamydial species. Further studies should focus on these or other protein antigens to identify the common targets of *C. trachomatis* and *C. suis* neutralizing antibodies and to evaluate the possible role of these antigens in vaccine development.

**References**


