Tissue microarray and immunohistochemistry as tools for evaluation of antibodies against *Chlamydia*-like bacteria

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Tissue microarray technology was used to establish immunohistochemistry protocols and to determine the specificity of new antisera against various *Chlamydia*-like bacteria for future use on formalin-fixed and paraffin-embedded tissues. The antisera exhibited strong reactivity against autologous antigen and closely related heterologous antigen, but no cross-reactivity with distantly related species.

INTRODUCTION

Several *Chlamydia*-like bacteria have recently been identified as potential emerging public health threats or pathogenic agents in animals. *Parachlamydia acanthamoebae*, *Protochlamydia naegleriaphila* and *Simkaniella negevensis* have been reported as possible aetiological agents of pneumonia in humans (Casson et al., 2008a; Corsaro & Greub, 2006; Friedman et al., 2006; Greub et al., 2003). *Parachlamydia* and related *Chlamydia*-like organisms have recently been reported in bovine abortion (Borel et al., 2007). *Waddlia chondrophila* has also been implicated as an abortigenic agent in bovines (Henning et al., 2002; Rurangirwa et al., 1999), and a zoonotic potential for *W. chondrophila* was suggested by an association of anti-*Waddlia* antibodies in women and sustained contact with animals (Baud et al., 2007). Infection may also occur following exposure to water colonized with infected free-living amoebae. Thus *Parachlamydia acanthamoebae* strain BN9 has been identified as a symbiont in free-living amoebae (Amann et al., 1997), whereas *Parachlamydia acanthamoebae* strain Hall’s coccus was identified within an amoeba isolated from a humidifier during investigation of an outbreak. *Candidatus* *Protochlamydia amoebophila*’ UWE25 was originally found in an *Acanthamoeba* isolate from a soil sample (Collingro et al., 2005). *Criblamydia sequanensis* was isolated from Seine river water using amoebal co-culture (Thomas et al., 2006) and *Neochlamydia hartmannellae* was detected in *Hartmannella vermiformis* isolated from the water conduit system of a dental care unit (Horn et al., 2000).

METHODS

*Acanthamoeba castellanii* (ATCC 30010) cultures were infected with *Parachlamydia acanthamoebae* strain Hall’s coccus or strain BN9 (ATCC VR-1476), *Candidatus* *Protochlamydia amoebophila* strain UWE25 (ATCC PRA-7), *Protochlamydia naegleriaphila* strain Knic, *Criblamydia sequanensis* strain CRIB-18, *S. negevensis* (ATCC VR-1471) and *W. chondrophila* (ATCC VR-1470). H. *vermiformis* strain BL was infected with *N. hartmannellae* (ATCC 50802). Uninfected *A. castellanii* and *H. vermiformis* cultures were prepared as negative controls.

Vero 76 (ATCC CRL-1587), Caco-2 (ATCC HTB-37) and HEP-2 (ATCC CCL-23) cells were infected with different human and animal chlamydial strains: Chlamydia pneumoniae strain Kajaini 6, Chlamydia abortus strain S26/3 (sheep abortion), Chlamydia psittaci strain T49/90 (psittacosis agent), Chlamydia pecorum strain 1710S (swine origin), Chlamydia trachomatis strain LGV 434 and Chlamydia suis strain S45/6 (swine isolate). Uninfected cell cultures of each cell line served as negative controls. Amoebal and cell pellets were fixed in formalin and embedded in paraffin as described elsewhere (Borel et al., 2006). A cell culture array block including two equally prepared sets of cell and amoebal pellets was created with the TMA Builder (Histopathology, http://www.histopath.hu) according to the manufacturer’s instructions. Briefly, the recipient paraffin block with 24 holes...
of 2 mm diameter each arranged in four columns and six rows was built using the TMA Builder. The whole cell pellets from the donor blocks were punched out with a Paraffin-Punch-Extractor and arrayed in a preformed recipient paraffin block according to the protocol. Four-micrometre sections were cut using a standard microtome.

For IHC, two commercially available chlamydial antibodies were used: a Chlamydiaceae family-specific mouse monoclonal antibody (mAb) directed against the chlamydial LPS (mLPS; clone ACI-P; Progen) and a Chlamydia-specific mouse mAb (IgG1) directed against recombinant Chlamydia trachomatis Hsp60 (clone A57-B9; Milan Analytica).

Polyclonal mouse antisera for the detection of various Chlamydia-like bacteria were produced previously and characterized by immunofluorescence and Western blotting (Casson et al., 2007). Optimization experiments for antigen retrieval and appropriate dilution of antisera for IHC were performed using infected amoebal and cell pellets. Primary antisera were then applied to the TMA as follows: (i) mLPS at a dilution of 1:50, (ii) anti-Hsp60 at a dilution of 1:1200, (iii) antisera against Parachlamydia acanthamoebae strain Hall’s coccus at a dilution of 1:6000, (iv) antisera against ‘Protoclamydia naegleriophila’ strain Knic at a dilution of 1:20 000 and (v) antisera against Parachlamydia acanthamoebae strain BN9, ‘Criblamydia sequanensis’ and W. chondrophila at dilutions of 1:40 000.

Detection was performed with a detection kit (ChemMate; Dako) according to the manufacturer’s instructions. Briefly, paraffin sections were deparaffinized in xylene and rehydrated through graded ethanol to water. Antigen retrieval was performed by 5 min enzyme digestion (Pronase; Dako) for the mLPS antibody, and repeated microwave heating (750 W for 10 min) in citrate buffer, pH 6.0 (Target Retrieval.
Solution, ChemMate; Dako), for the antisera against *Chlamydia*-like bacteria. Primary antibodies were incubated for 1 h. For inhibition of endogenous peroxidase activity, the slides were immersed in peroxidase-blocking solution (Dako) for 5 min at room temperature. Negative and positive controls for each section were included as described elsewhere (Borel et al., 2006).

**RESULTS AND DISCUSSION**

The mLPS and anti-Hsp60 antibodies, as well as the antisera against the different *Chlamydia*-like bacteria, did not exhibit cross-reactivity against uninfected cell or amoebal pellets (Fig. 1a, i). Table 1 shows the antibody titres and cross-reactivities of the different chlamydial antibodies with *Chlamydiaceae* and *Chlamydia*-like bacteria, as determined by IHC on the TMA. In general, the antibodies with titres and cross-reactivities of the different chlamydial amoebal pellets (Fig. 1a, i). Table 1 shows the antibody titres and cross-reactivities of the different antibodies with *Chlamydiaceae* and *Chlamydia*-like bacteria. The mLPS antibody reacted with the *Chlamydiaceae* strains as expected (Fig. 1g), but not with *Chlamydia*-like bacteria. The same was observed for the anti-Hsp60 antibody (Fig. 1h), except for some cross-reactivity to the pellet infected with *N. hartmannellae*.

Antisera against *Parachlamydia acanthamoebae* strain Hall’s coccus and *W. chondrophila* have already been applied successfully to formalin-fixed and paraffin-embedded bovine placenta specimens and resulted in the first report of *Parachlamydia* in bovine abortion (Borel et al., 2007). The antibody against *Parachlamydia acanthamoebae* strain Hall’s coccus has also been successfully applied to lung samples from mice experimentally infected with *Parachlamydia acanthamoebae*. IHC results of lungs correlated well with histopathological lesions and real-time PCR (Casson et al., 2008).

Reactivity to LPS was not observed in any *Chlamydia*-like bacteria tested. This suggests that these species do not have a LPS or possess a truncated LPS, as shown for *Candidatus* Protochlamydia amoebophila* UWE25 (Horn et al., 2004). Thus these new agents will not be detected by routine diagnostics when using an antibody directed against chlamydial LPS. Knowledge of the presence of heat-shock proteins in *Chlamydia*-like bacteria is scarce. Nevertheless, some cross-reactivity of the anti-Hsp60 antibody could be observed in the *N. hartmannellae*-infected amoebal pellet, but not in *H. vermiformis* pellets, suggesting the presence of a heat-shock protein-like structure in this species.

In conclusion, TMA technology in combination with IHC is a useful tool for testing the specificity of antibodies for their future use on formalin-fixed and paraffin-embedded tissues. Possible cross-reactivity of antibodies in closely

Table 1. Dilutions and cross-reactivities of different antibodies with *Chlamydiaceae* and *Chlamydia*-like bacteria, as determined by IHC on TMA

<table>
<thead>
<tr>
<th>Species studied</th>
<th>Parachlamydia acanthamoebae strain Hall’s coccus</th>
<th>Parachlamydia acanthamoebae strain BN9</th>
<th>‘Protochlamydia naegleriophila’ strain Knic</th>
<th>‘Criblamydia sequanensis’</th>
<th>Waddlia chondrophila LPS</th>
<th>Hsp60</th>
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<td>1:40 000</td>
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<td>0</td>
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<tr>
<td>‘Protochlamydia naegleriophila’ strain Knic</td>
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<td>0</td>
<td>1:20 000</td>
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<td>0</td>
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<tr>
<td>‘Candidatus Protochlamydia amoebophila’ strain UWE25</td>
<td>1:6000*</td>
<td>0</td>
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<td>‘Criblamydia sequanensis’</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>1:50</td>
</tr>
</tbody>
</table>

*These results were interpreted as questionable, as they were not confirmed in another experiment.
related species should be considered when investigating human or animal tissues with these antisera by IHC.

ACKNOWLEDGEMENTS

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REFERENCES


