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Recombinant major outer membrane protein (MOMP) of *Chlamydophila abortus*, *Chlamydophila pecorum*, and *Chlamydia suis* as antigens to distinguish chlamydial species-specific antibodies in animal sera

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Abstract

Recombinant major outer membrane proteins (rMOMP) of *Chlamydophila* (*Ch.*), *Chlamydia* (*C.*) *abortus*, *Ch. pecorum*, and *C. suis* were used as antigens to distinguish chlamydial species-specific antibodies in (i) immune sera from six rabbits and three pigs raised against native purified elementary bodies, (ii) serum samples from 25 sows vaccinated with *Ch. abortus*, and (iii) 40 serum samples from four heifers experimentally infected with *Ch. abortus*. All post-exposition sera contained chlamydial antibodies as confirmed by strong ELISA seroreactivities against the chlamydial LPS. For the rMOMP ELISA mean IgG antibody levels were at least 5.8-fold higher with the particular rMOMP homologous to the chlamydial species used for immunisation or infection than with heterologous rMOMPs (*P* < 0.001). Preferential rMOMP ELISA reactivities of sera were confirmed by Western blotting. The results suggest that the entire chlamydial rMOMP could provide a species-specific serodiagnostic antigen.

Keywords: *Chlamydophila abortus; Chlamydophila pecorum; Chlamydia suis; Recombinant MOMP; serology; Species-specific antibodies*

1. Introduction

Serodiagnostic tests are important for the monitoring of animal chlamydial infections. To date, the serological assays performed predominantly comprise screening assays like the complement fixation test (CFT) and the enzyme-linked immunosorbent assay (ELISA). Reactions of both assays are as a rule group-specific due to the antigenic cross-reactivity of the chlamydial lipopolysaccharide (LPS) which is present in all chlamydiae (Everett, 2000). Thus, those tests fail to identify the causative chlamydial species. The 40 kDa chlamydial major outer membrane protein (MOMP) is known to possess serotype-, subspecies-, species-, and genus-specific antigenic determinants (Conlan et al., 1988; Salti-Montesco et al., 1997).
In the ensuing research for more discriminatory veterinary serodiagnostic tests, Kaltenboeck et al. (1997) used synthetic peptide antigens deduced from the variable segments VS 2 and VS 4 of the Chlamydo-
philia (Ch.) abortus MOMP for the detection of species-specific antibodies in ovine and bovine sera. Recently, an indirect ELISA using recombinant fragments of the polymorphic outer membrane proteins (POMP) of Ch. abortus as antigens was described which allows to differentiate Ch. abortus antibodies in ovine sera (Longbottom et al., 2002). A serodiagnostic test combining species-specific recombinant proteins from different chlamydial species could have the inherent capability of discriminating serologically infections on the species level. The aim of this work was to determine whether recombinant MOMPs (rMOMP) of Ch. abortus, Ch. pecorum, and Chlamydia (C.) suis show potential as serodiagnostic antigens to distinguish chlamydial species-specific antibodies in animal sera.

2. Materials and methods

The cloning and expression of the ompA gene of Ch. abortus, Ch. pecorum, and C. suis in Escherichia (E.) coli strain LMG194 (Invitrogen, NV Leek, Netherlands) was described previously (Hoelzle et al., 2003). Three serum panels were examined. Group 1: Experimental sera from six New Zealand White rabbits (2–3 kg) and three cross-bred pigs (6–8 weeks of age) which were immunised with purified elementary bodies (EB) of either Ch. abortus, Ch. pecorum, or C. suis. Immunisations were conducted under registration numbers 156/2002 (rab-
bits) and 4.8/8746-1/161/02 (pigs) in compliance with the legal prescriptions. Group 2: Sera from 25 sows (German landrace) taken prior to and 7 weeks after vaccination with Ch. abortus (Knitz et al., 2003). Group 3: Sera from four heifers subjected to experimental intrauterine infection with Ch. abortus (Wittenbrink et al., 1993). From each heifer two sera were taken prior to infection and ten sera were taken at weekly intervals from week 2 until week 11 after infection. Sera were stored in aliquots at −20 °C. The indirect ELISA and Western blot analyses using chlamydial LPS or rMOMPs as antigens were described previously (Hoelzle et al., 2003; Knitz et al., 2003). Statistical analysis of ELISA net optical density (OD405) values was performed with SigmaStat® Software Version 3.0 (SPSS Inc., Chicago, IL, USA). Median net OD values were compared by Kruskal–Wallis ANOVA on ranks. Statistical significance was set at \( P < 0.05 \).

![Fig. 1. Titration of the porcine Ch. abortus antiserum against standardised concentrations (1.2 μg/ml) of the rMOMP of Ch. abortus, Ch. pecorum, and C. suis. Each datum point is the mean of three OD405 values. Error bars indicate standard deviations. Sensitivity is defined as change of ELISA reactivity (dR) in response to change of serum concentration (dC). Data of the sensitivity plot were calculated as dR/dC and constructed as a 3rd order polynomial regression.](image-url)
3. Results

Immune sera from rabbits and swine were titrated against predetermined equal concentrations of homologous and heterologous rMOMPs. Fig. 1 shows a typical example of the effect of different rMOMPs on the dose-response curves from the porcine Ch. abortus immune serum. The homologous titration (Ch. abortus antiserum versus Ch. abortus rMOMP) revealed maximum OD values. Analysis of the linear portion of the titration curves showed a significant linear regression between serum dilution and the OD values for the homologous reactions ($r = -0.99; P < 0.001$). Sensitivity of the homologous seroreactivity yielded a peak value at a serum dilution between 1:32 and 1:64. At these serum dilutions cross-reactions between the Ch. abortus antiserum and heterologous rMOMPs were minimal and comprised a maximum of 12.4% on average of the homologous reactions. Therefore, a serum dilution of 1:32 was chosen for subsequent investigations. Compatible results were obtained from titrations of other immune sera against the different rMOMP antigens (data not shown). The inter-assay and intra-assay reproducibility of the ELISA given as coefficients of variation between triplicate assays were 0.11 and 0.09, respectively.

Results of repeated testings of rabbit immune sera in single dilution assays against chlamydia LPS and rMOMPs are shown in Fig. 2. Each antiserum revealed maximum reactivity with the homologous rMOMP. Taking the mean net OD values of homologous reactions as 100%, the percentage of serological cross-reactivity with heterologous rMOMPs was 17.2% on average. Net OD values with the homologous rMOMP were significantly different from net OD values with heterologous rMOMPs ($P < 0.001$; Kruskal–Wallis ANOVA on ranks). Besides the strong reactions with the homologous rMOMP, rabbit immune sera consistently showed strong seroreactivity with the chlamydial LPS antigen despite using a six-fold higher serum dilution (1:200 in the LPS-ELISA instead of 1:32 in the rMOMP ELISA). ELISA testing of porcine immune sera revealed compatible results (data not shown).

Serum samples from 25 sows vaccinated with Ch. abortus and serum samples from four heifers after experimental uterine infection with Ch. abortus exhibited strong seroreactivities with the chlamydial LPS antigen (mean net OD: 1.11 ± 0.46, sows; 0.83 ± 0.07, heifers). Comparative rMOMP seroreactivities matched the pattern of the rabbit Ch. abortus antiserum given in Fig. 2A. Mean net OD values of sera from vaccinated sows and experimentally infected heifers against Ch. abortus rMOMP were 1.30 ± 0.30 and 0.79 ± 0.08, respectively and were significantly different from net OD values against Ch. pecorum rMOMP (0.42 ± 0.11; 0.31 ± 0.04) and C. suis rMOMP (0.19 ± 0.05; 0.19 ± 0.03; $P < 0.001$).

![Fig. 2. IgG antibody responses of rabbit immune sera raised against native EB of Ch. abortus (A), Ch. pecorum (B), and C. suis (C). ELISA reactivities were determined in 1:200 serum dilutions (LPS-ELISA) and 1:32 dilutions (rMOMP ELISA). Bars indicate mean net OD values of 10 independent ELISA runs. Error bars indicate standard deviations. Cutoff values were 0.178 (rMOMP ELISA, dashed line) and 0.155 (LPS-ELISA, not plotted).](image-url)
Cutoff values were 0.26 (LPS ELISA) and 0.27 (rMOMP ELISA).

With Western blotting, pre-immunisation sera from rabbits and pigs showed no reactions with rMOMPs, whereas immune sera revealed specific reactions with the homologous rMOMP (Fig. 3). Reactions to the E. coli LMG194 control antigen were not detected and cross-reactions to heterologous rMOMPs, if present, were only faint. Sera from Ch. abortus-vaccinated sows and sera from experimentally Ch. abortus-infected heifers showed strong reactions with Ch. abortus rMOMP and no reactions with heterologous rMOMPs (data not shown).

4. Discussion

Recombinant antigens are to date not widely used in veterinary chlamydial serology and there is no general agreement as to which proteins are best for sensitivity and specificity (Longbottom et al., 2002). The 40 kDa MOMP is the immunodominant antigen at the chlamydial surface known to induce a strong humoral immune response (Caldwell et al., 1981). In animal chlamydial infections typical patterns of the antibody response are a strong seroreactivity with the homologous MOMP and only a weak or no reactivity with the heterologous MOMP (Mondesire et al., 1989; Baghian et al., 1990; Jensen et al., 1993). These observations prompted us to investigate whether a serological assay using rMOMPs from three veterinary important chlamydial species as antigens is capable of discriminating chlamydial species-specific antibodies in animal sera. Since the number and distribution patterns of species-specific epitopes on the MOMP of animal chlamydiae are, for a greater part, undefined and since our knowledge about MOMP epitopes which are recognised consistently by sera of naturally infected animals is rather poor, our approach was to use the entire MOMP in a recombinant antigen and thus to provide the whole spectrum of epitopes for antibody recognition. Hereby we were able to demonstrate significant differences in ELISA responses between sets of serum samples from animals immunised or infected experimentally with representatives of three veterinary significant chlamydial species. In all tests maximum ELISA seroreactivity was measured with the rMOMP homologous to the particular chlamydial species used for immunisation or infection, and ELISA results were consistently confirmed by Western blot analyses. These results emphasise a high antigenic diversity of the MOMP of the chlamydial species investigated. Compatible with this, cross-reactive ompA gene sequences between Ch. abortus, Ch. pecorum, and C. suis were not detectable in Southern blotting studies using the entire ompA genes as a probe (Hoelzle et al., 2000). Although hitherto unspecified genus-specific antigenic sites responsible for cross-reactivity also reside in MOMP (Hoelzle et al., 2003), the level of serological cross-reactivity between the rMOMPs as measured by ELISA did not exceed 17.2% of the corresponding homologous positive values. This result is consistent with data from
the literature that genus-specific MOMP epitopes are not or only severely immunoaccessible (Batteiger et al., 1986; Zhang et al., 1987; Conlan et al., 1988). Compatible with this, polyclonal monospecific antibodies raised against SDS-PAGE separated MOMP or against rMOMP react strongly with either homologous or heterologous MOMPs, corroborating that cross-reactive, presumably genus-specific MOMP epitopes are merely immunoaccessible after being removed from the intact outer membrane of the chlamydial cell (Fig. 3; Baghian et al., 1990; Hoelzle et al., 2003).

Since our rMOMPs were expressed as E. coli inclusion bodies and purified under strongly denaturing conditions (8.0 M urea, heating in 1.0% SDS), we agree with data from the literature that the species-specificity of the humoral immune response in animal sera is primarily not dependent on the conformational nature of the native MOMP (Conlan et al., 1988; Baghian et al., 1990; Kaltenboeck et al., 1997). Cross-reactive antibodies recognise predominantly the family-specific LPS, irrespective of the chlamydial species which induced the humoral immune response (Ossewaarde et al., 1994). All sera tested in our study revealed strong seroreactivities with the chlamydial LPS. This in turn justifies the conclusion that the binding of cross-reactive antibodies like LPS antibodies or antibodies against cross-reactive epitopes on chlamydial surface proteins other than MOMP (e.g. 60 kDa cysteine-rich protein, Watson et al., 1994), could mask the seroreactivity of antibodies against species-specific MOMP epitopes if chlamydial whole cells or extracts containing cross-reactive moieties are used as antigens (Bessho, 2000; Ossewaarde et al., 1994). Therefore, a serological assay based on recombinant purified proteins like rMOMP apparently excludes the binding of cross-reactive antibodies and, thus, is expected to improve specificity.

In conclusion, this is the first report on the suitability of entire chlamydial rMOMPs as antigens in veterinary serodiagnosis. Due to the species-specific seroreactivity patterns, our antigens may be valuable for developing a diagnostic test which could allow to serologically identify the infecting chlamydial species. In order to confirm the diagnostic utility of our rMOMPs, further investigations on sera from natural infections, in particular from animals with verified asymptomatic chlamydial mono- and mixed infections are required.

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References


