Antigenic Diversity of *Chlamydia psittaci* of Mammalian Origin Determined by Microimmunofluorescence

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A group of twenty-five isolates of *Chlamydia psittaci* representing at least seven different biotypes of bovine, ovine, caprine, equine, feline, porcine, and guinea pig origin were immunotyped by an indirect microimmunofluorescence test. Different groups of chlamydia-free BALB/c mice received two weekly intravenous inoculations with chicken embryo-propagated, partially purified elementary bodies of each strain. Antisera for immunotyping were collected 4 days after the first inoculation and 3 to 4 days after the second inoculation and tested for antichlamydial immunoglobulin M and immunoglobulin G antibodies by the indirect microimmunofluorescence test with cell culture-propagated, partially purified homologous and heterologous antigens. Nine immunotypes of *C. psittaci* were distinguished. The correlation between immunotypes and biotypes was close, and a pattern of either disease or host specificity could be associated with each immunotype. Most immunotypes identified induced crossed-reacting antibodies against each other, but no significant cross-reactions were observed with elementary bodies of the mouse pneumonitis strain of *C. trachomatis*. Findings from this study should provide the necessary background for the rational selection of prototype strains of *C. psittaci* for further antigenic analysis at the molecular level.

The genus *Chlamydia* includes a large group of pathogens that can multiply only within the cytoplasm of living animal cells (15, 17). Based on their susceptibility to sulfadiazine, their production of glycogen, and the presence of a species-specific antigen, two species are currently distinguished (4, 5, 18). Further subdivisions within each species are based on properties that do not define the species. *Chlamydia trachomatis* infects only man and mice, and all known strains were subdivided into three biotypes based on their natural host and biological properties determining their interaction with cultured cells (9, 17). In addition, this species was also subdivided into 15 immunotypes through strain-specific protein antigens found on the chlamydial envelope and usually demonstrated by indirect immunofluorescence (9, 16).

*Chlamydia psittaci*, the second species of the genus, comprises a large collection of strains and exhibits broad host ranges and pathogenic potentials (31, 33). Several efforts have been made to differentiate this highly heterogeneous species (8, 10, 14, 22–28, 39), but only limited success has been achieved. Schachter et al. (25, 26) differentiated several isolates of *C. psittaci* of ovine and bovine origin into two broad antigenic groups based on a plaque reduction test, but a few strains of similar origin could not be typed. Antisera against these strains did not neutralize infectivity of several strains of avian origin. More recently, Spears and Storz (27) studied chlamydial strains isolated from a broader range of animal hosts. Based on inclusion morphology and response to different treatments in cultured cells, eight biotypes of *C. psittaci* were established, but the usefulness of this classification scheme remained limited because considerable experience is required and the process is very laborious.

The purpose of this investigation was to immunotype different biotypes of *C. psittaci* of mammalian origin by a modification of the indirect microimmunofluorescence (IMIF) test of Wang (37).

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MATERIALS AND METHODS

**Chlamydial strains.** Twenty-five strains of *C. psittaci* representing at least seven biotypes of bovine, ovine, caprine, porcine, feline, and guinea pig origin were used throughout this investigation. The origin of each isolate, together with other relevant information, is summarized in Table 1.

**Chlamydial antigens for mouse inoculations.** Chlamydial antigens used for inoculation of mice consisted of partially purified elementary body (EB) suspensions. Briefly, the different chlamydial strains were propagated in chicken embryos as described previously (28). Heavily infected yolk sacs were homogenized in sucrose phosphate buffer (1), and the suspension was centrifuged at 200 × g for 5 min at 4°C. The chlamydiae present in the supernatant were pelleted at 12,000 × g for 1 h at 4°C and suspended in 0.01 M phosphate-buffered saline (PBS) (pH 7.1).

Partial purification was accomplished by centrifugation at 40,000 × g for 1 h at 4°C through a solution of 35% Renografin (diatrizoate meglumine and diatrizate sodium, 76% for injection; E. R. Squibb & Sons, Princeton, N.J.) in PBS. The partially purified chlamydial EBs were washed once and suspended in a final volume of 10 ml of PBS per yolk sac. Antibodies that were not used immediately for mouse inoculations were stored at −70°C.

**Chlamydial antigens for IMIF.** Chlamydial antigens used in the IMIF test consisted of L-cell-propagated, partially purified EB suspensions. Briefly, cultures of L cells were infected by a modification of the procedure described by Lee (11). Confluent monolayers grown in 60-mm petri dishes were inoculated with 10⁻² dilutions of clarified yolk sac homogenates with a centrifugal force of 2,000 × g for 30 min at 37°C. The infected cells were incubated at 37°C in culture medium containing 2 μg of cycloheximide per ml, and chlamydiae were harvested 48 to 72 h later after freezing and thawing the monolayers. Partial purification of chlamydial EB was accomplished by the procedure described above. Yields obtained from 24 to 36 petri dishes were suspended in
1 to 2 ml of PBS containing 0.12% bovine serum albumin and sonicated for 15 to 20 s at a power setting of 40 with a Branson cell disruptor. All antigens were divided into aliquots of 0.25-ml amounts and stored at -70°C.

Production of antisera in mice. Different groups of chlamydia-free BALB/c mice (10 to 20 mice per group) received two weekly intravenous inoculations (0.5 ml per mouse per inoculation) of the chicken embryo-propagated, partially purified chlamydial antigens. Immunoglobulin M (IgM)-rich antisera were collected 4 days after the first inoculation, and IgG-rich antisera were collected 3 to 4 days after the second inoculation. All serum samples were stored at -70°C.

IMIF test. The IMIF test was performed by a modification of the procedure described by Wang (37). Briefly, small amounts of each cell-propagated antigen were plated on 12-well Teflon-coated slides (Cell-Line Associates Inc., Newfield, N.J.) with a 20-μl Eppendorf pipette. The antigens were arranged in groups of four per well. By placing a different set of antigens on the top and bottom row of each slide, eight different antigens could be tested with one serum on each slide.

After a 30-min period of air drying at room temperature, the slides were fixed in cold formaldehyde (4% in PBS [pH 7.2]) for 5 min, and 15 μl of the proper antiserum dilution (twofold dilutions from 1:8 to 1:256) were used, followed by 15 μl of the working conjugate dilution (fluorescein isothiocyanate-conjugated sheep anti-mouse IgG heavy and light chain specific or fluorescein isothiocyanate-conjugated goat anti-mouse IgM μ chain specific; U.S. Biochemical Corporation, Cleveland, Ohio) in PBS with rhodamine-conjugated bovine albumin (Difco Laboratories, Detroit, Mich.) as a counterstain. The microscopic examination was performed with a Zeiss epifluorescence microscope equipped with an x40 oil immersion lens. The highest serum dilution giving specific fluorescence associated with EBs was recorded as the endpoint titer.

RESULTS

The IgM-specific antibody response of mice was relatively low at 4 days postinoculation. However, a pattern of antigenic relationships emerged, because strains of the same biotype showed extensive cross-reactions. Results obtained with the IgG-specific IMIF test are summarized in Table 2. In this instance, the specificity of the immune reaction within a given biotype was maintained, but the extent of cross-reactions between some serotypes increased. Based on the IgG antibody responses of mice, nine immunotypes of C. psittaci were distinguished. An overall scheme of antigenic relationships among the different immunotypes identified was established (Fig. 1). With this information, additional chlamydial strains of bovine, caprine, and equine origin were immunotyped in one-way tests with antisera in nine strains representing the different immunotypes identified (Table 3).

With one exception (serotype 6), the different immunotypes of C. psittaci identified by the IMIF test were numbered according to the predominant biotype which they represented. Immunotype 1 comprised chlamydial strains previously classified as biotype 1 plus four chlamydial strains of bovine, caprine, and equine origin whose biotype has not been determined previously. Immunotypes 2, 3, 5, and 8 comprised strains of biotypes 2, 3, 5, and 8. Immunotype 4 included a chlamydial strain of biotype 4 plus a porcine isolate of unknown biotype. Immunotype 6 was
### Table 2: Microimmunofluorescence reaction of different blots of C. psittaci mediated by mouse IgG antibodies

<table>
<thead>
<tr>
<th>Serum No.</th>
<th>128</th>
<th>64</th>
<th>32</th>
<th>16</th>
<th>8</th>
<th>4</th>
<th>2</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Serum 2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Serum 3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Serum 4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

ND: Not determined.
represented by a chlamydial strain of biotype 4 together with one porcine and one bovine isolate, both of undetermined biotype. Immunotype 7 included the strain of the feline pneumonitis vaccine (biotype 7) and the Z-10 strain reported by bovine origin and undetermined biotype. Immunotype 9 included one ovine isolate whose biotype has not been determined.

The yields of EBs obtained from cell cultures infected with the different chlamydial strains varied considerably. Variations in their degree of cell association were also observed, despite being cultivated and partially purified under the same conditions. Consequently, the IMIF reaction was more difficult to read with some antigens than with others. To diminish this subjective effect, a 50% difference in titer among strains of the same biotype was not considered significant.

**DISCUSSION**

The IMIF test differentiated 25 strains of *C. psittaci* of mammalian origin into nine immunotypes. A good correlation was observed between immunotypes and biotypes, indicating that strains with unique biological properties also have unique antigenic composition.

This proposed immunotyping scheme should be expanded to include other chlamydial strains, especially those of avian origin. Although none of the *C. psittaci* immunotypes identified in this study cross-reacted significantly with the mouse pneumonitis strain, antigenic relationships with other strains of *C. trachomatis* should also be explored.

Our findings are in agreement with previous reports on the antigenic and genomic relationships of limited numbers of ovine strains of *C. psittaci*. Eb and Orfila (7), using the IMIF test, differentiated six ovine abortion strains of similar antigenic makeup from the ovine arthropathogenic strain LW-679. More recently, McLennan and colleagues (14), using DNA restriction endonuclease analysis, identified unique DNA fragments common to eight ovine chlamydial abortion strains which differed to some extent from those of a single isolate obtained from polyarthritis lamb in Scotland.

The different immunotypes identified in our investigation apparently have some degree of disease and host specificity. The intestinal mucosa emerges as a common site of infection. Immunotype 1 includes strains isolated from ruminants affected with abortions, seminal vesiculitis, pneumonia, and clinically inapparent intestinal infections. The pathogenic potential of most of these isolates had been confirmed experimentally (31, 33). Two isolates from horses, one from the lung and one from synovial fluid, are also identified as immunotype 1. However, their pathogenic properties have not been assessed experimentally. Since none of the other strains classified as immunotype 1 has arthropathogenic properties for cattle or sheep, and since all arthropathogenic strains have uniquely distinguished features, more equine isolates should be studied to determine whether they are indeed related to strains from ruminants. It could be that the isolate recovered from a foal with polyarthritis was spurious.

Immunotype 2 also includes strains isolated from ruminants, but the disease association of this group of strains is conjunctivitis, polyarthritis, encephalitis, and enteritis under natural and experimental conditions (31, 33).

Immunotypes 3 and 9 are represented by chlamydial isolates which appear to be part of the intestinal flora of cattle and sheep. The pathogenic potential of these isolates has not been explored experimentally.

Immunotypes 4, 5, and 6 represent porcine strains associated with polyarthritis or generalized infections, clinically inapparent intestinal infections, and abortion or pneumonia, respectively. A single isolate from bovine pneumonia is also identified as immunotype 6. The pathogenicity of these strains has not been studied experimentally.

Immunotype 7 includes the chlamydial strain used in the live feline pneumonitis vaccine and an isolate presumably

**TABLE 3. Results of a one-way immunotyping of four *C. psittaci* isolates mediated by mouse IgG antibodies in the IMIF test**

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Cross-reaction with following immunotype (antisera)*</th>
<th>Immunotype identified</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Type 1 (B-577)</td>
<td>Type 2 (LW-623)</td>
</tr>
<tr>
<td>Equine pneumonia</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Bovine pneumonia L-1</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Bovine pneumonia L-14</td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td>Goat abortion</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

* Cross-reaction as percentage of the type antiserum titer with its homologous antigen. A value of zero indicates <6% or no reaction of serum at 1:8 dilution.
cultured from a calf with pneumonia (strain Z-10). Whether a common chlamydial antigenic makeup may be associated with respiratory infections in cats and cattle remains to be proven. In some areas of this country, it has been common practice to vaccinate cattle with the live feline pneumonitis vaccine (Fromm Laboratories, Grafton, Wis.). It is not known whether the bovine isolate classified as immunotype 7 comes from a calf of a herd vaccinated with the feline pneumonitis strain or whether it is a naturally occurring pneumopathogenic strain from calves.

While some of the immunotypes identified induced antibodies which reacted only with the homologous strains (immunotypes 5 and 8), most of the other immunotypes induced cross-reacting antibodies. Notably, immunotype 2 strains did not seem to induce good antibody responses in mice, and cross-reactions of the same magnitude as the homologous low-titered reactions were frequently present with antigens of different immunotypes.

The molecular basis for the specific antigenic relationships observed was not determined. However, Caldwell et al. (2, 3, 6) have demonstrated that a major outer membrane protein of molecular size of approximately 39.5 kilodaltons is responsible for the immunotype specificity observed in the genus *Chlamydia*. Antibodies against purified major outer membrane protein from several strains of *C. trachomatis* and the meningocoxnis strains of *C. psittaci* reacted with the same specificity as whole EBs in the IMIF test (3).

The *Chlamydia*-specific lipopolysaccharide LPS antigen is also found in the envelope of the EBs (21), but its effect on the antigenic relationships observed in the IMIF test appears to be insignificant. Wang (37) suggested that the early mouse antibodies used in the IMIF test contain not only type-specific but also genus-specific antibodies. However, the chlamydial lipopolysaccharides do not seem to be readily available on the surface of untreated or paraformaldehyde-fixed EBs (29, 38).

The findings in our investigation should provide the necessary background for the rational selection of prototype strains of *C. psittaci* to be used in more refined studies involving the interaction of antibodies with purified chlamydial antigens and the production of monoclonal antibodies of defined specificity.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant AI08420-13 from the National Institute of Allergy and Infectious Diseases; by special research grants 80-CSRS-02-105 and 84-CSRS-2-2445 from the U.S. Department of Agriculture; by financial support through Salsbury Laboratory, Charles City, Iowa, and Fromm Laboratories, Grafton, Wis.; and by a scholarship from the Universidad Nacional Autonoma, Mexico City, Mexico.

This paper contains parts of a dissertation presented by the senior author (J. A. P. -M.) to the graduate school of Louisiana State University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

LITERATURE CITED


