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Antibody Response to Epitopes of Chlamydial Major Outer Membrane Proteins on Infectious Elementary Bodies and of the Reduced Polyacrylamide Gel Electrophoresis-Separated Form

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Approximately 60% of the outer membrane of chlamydial elementary bodies (EBs) consists of the major outer membrane protein (MOMP) that has structural and metabolic functions. The antigenic properties of MOMPs from mammalian strains of serovars 1 and 2 and an avian strain of *Chlamydia psittaci* were analyzed. Polyclonal-monospecific antisera (PMAs), one monoclonal antibody (MAb), and polyclonal antisera (PAs) were produced against reduced polyacrylamide gel electrophoresis-separated MOMPs and against infectious EBs. Three PMAs and the MAb, which were induced by reduced polyacrylamide gel electrophoresis-separated MOMPs, reacted strongly in Western blot (immunoblot) assays with MOMPs of serovar 1 and 2 strains as well as with that of the avian strain 6BC, and two of these PMAs reacted weakly (dilution, 1:20) with the MOMP of strain LGV-2. The third PMA and the MAb against the MOMP of the serovar 2 strain did not react with the MOMP of LGV-2. Four PAs were produced against infectious EBs of the serovar 1 strain. One of these PAs reacted with the homologous MOMP and that of the avian strain 6BC but did not recognize MOMPs of other chlamydial strains. Three of the PAs reacted with MOMPs of homologous strains only and failed to recognize MOMPs of avian, serovar 2, and LGV-2 strains. Five PAs induced against infectious EBs of the serovar strain 2 reacted only with the MOMPs of the homologous strains and failed to recognize MOMPs of other strains of chlamydiae. Consequently, MOMPs of *C. psittaci* strains possess genus-, species-, and serovar-specific epitopes whereby the immune response to serovar-specific epitopes of MOMP predominate when infectious EBs are used for immunization.

Members of genus *Chlamydia* are obligate intracellular parasites. This genus consists of three species, *C. trachomatis*, *C. pneumoniae*, and *C. psittaci*, that are major pathogens for humans (*C. trachomatis* and *C. pneumoniae*) and animals (*C. psittaci*). Strains of biovar trachoma cause infections of the eyes, genital tracts, and respiratory organs. The lymphogranuloma serotypes (L1 to L3) cause a more invasive form of infection, lymphogranuloma venereum (9). Although *C. psittaci* strains infect mainly animals, humans may become infected as well (22). Strain TWAR of *C. pneumoniae* is a primary human respiratory pathogen (8).

C. psittaci strains are heterogeneous, and many strains have not been classified. Based on inclusion morphology and the effects of cytoactive agents, some mammalian strains of *C. psittaci* were grouped into eight biovars (19). This biotyping was further substantiated through serotyping techniques (17). Strains of serovar 1 are principally associated with intestinal infections and abortions, while strains of serovar 2 cause polyarthritis, encephalitis, and conjunctivitis in ruminants (17, 19, 22). Avian strains of *C. psittaci* cause respiratory problems and diarrhea in birds (22). Affected birds may transmit the infection to human subjects.

Classification of mammalian strains of *C. psittaci* into nine serovars and those of *C. trachomatis* into three biovars and different serotypes is based on indirect immunofluorescence techniques with polyclonal antisera (PAs) against whole elementary bodies (EBs) (17, 25) or on monoclonal antibodies (MAbs) against as yet undefined chlamydial antigens (26). Although this technique is reliable for serotyping chlamydiae, the use of PAs does not specify the target antigens. Recent investigations used MAbs raised against specific chlamydial proteins. The major outer membrane protein (MOMP), which was estimated to make up 60% of the proteins of the chlamydial outer membrane, was successfully used in classifying *C. trachomatis* in terms of genus, species, and serovar specificities. This 39- to 41-kilodalton protein was also found to be responsible for metabolic functions and the structural integrity of EBs (2–6, 14, 20, 27, 29).

Recently, the MOMP genes of some *C. psittaci* strains and *C. trachomatis* serovars were sequenced (2, 18, 27, 28). These sequences revealed that, except for four variable regions, significant portions of the MOMP genes are conserved. The variable domains were involved in the neutralization of EB infectivity, in serotype specificity (2, 16, 29), as well as in the pathogenicity of chlamydiae (2, 23).

The purpose of this study was to analyze by immunoblotting the reactivities of an MAb, polyclonal-monospecific antisera (PMAs), and PAs produced against either reduced polyacrylamide gel electrophoresis (PAGE)-separated MOMPs or infectious EBs of *C. psittaci* strains. A second aim was to determine the presence of genus-, species-, and serovar-specific epitopes on the MOMPs.

MATERIALS AND METHODS

Chlamydial strains and their propagation. Serovar 1 and avian strains of *C. psittaci* were grown in large quantities in L cells. Georgia bovine kidney (GBK) cells were more efficient for the propagation of serovar 2 strains as well as some other chlamydial strains. The GBK monolayers were grown in minimal essential medium with 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; Sigma

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TABLE 1. Immunoblot reactivities of antisera produced against infectious chlamydial EBs and reduced PAGE-separated MOMPs

Antiserum	Strain	Antigen	Rabbit no.	Reactivities of antisera against MOMPs of strain[a]:				
				B577	6BC	FC-Stra	LW-613	LGV-2
PMA	B577	MOMP	1MB	2,560	2,560	2,560	2,560	20
PMA	LW-613	MOMP	2ML	1,280	1,280	2,560	2,560	40
PMA	FC-Stra	MOMP	9MF	5,120	2,560	2,560	2,560	0
MAb	FC-Stra	MOMP		4	8	2	1	0
PA	LW-613	EB	1PL	−	−	+	+	−
PA	LW-613	EB	2PL	−	−	+	+	−
PA	LW-613	EB	3PL	−	−	+	+	−
PA	LW-613	EB	4PL	−	−	+	+	−
PA	LW-613	EB	25PL	−	−	+	+	−
PA	B577	EB	1PB	+	−	−	−	−
PA	B577	EB	FA-1PB	+	−	−	−	−
PA	B577	EB	3PB	+	+	−	−	−
PA	B577	EB	56PB	+	−	−	−	−

[a] Values are titers of serum that reacted in the immunoblot. −, No reaction; +, positive reaction.

Chemical Co., St. Louis, Mo.) supplemented with 10% heat-inactivated fetal bovine serum, 200 μg of streptomycin per ml, 25 μg of vancomycin per ml, and 2.5 μg of fungizone per ml. One day before infection, cells cultured in 150-cm^2 tissue culture flasks were trypsinized and split 1:2. The next day the medium was decanted, and the cells were infected with yolk sac-propagated EBs suspended in sucrose-phosphate-glutamic acid (SPG; pH 7.2) supplemented with 2% fetal bovine serum and antibiotics. The flasks were rocked on a platform at 37°C for 4 h. SPG was replaced with fresh medium, and the flasks were incubated at 37°C. A persistent infection was established, and EBs were collected 7 to 14 days postinfection and at wipeout points (13).

Purification of EBs. At the wipeout points, the supernatants of persistently infected cultures were pooled and centrifuged at 500 × *g* for 10 min at 4°C. The pellets were suspended in SPG, briefly sonicated, and centrifuged again as described above. The two supernatants were pooled, and the EBs were pelleted at 30,000 × *g* in a centrifuge (refrigerated superspeed; Sorvall RC-5B; Du Pont Instrument) for 1 h. Pellets were suspended in SPG to approximately 1/10 of the original volume. They were then partially purified by layering them over 10 ml of 35% (vol/vol) Renografin solution (diatrizoate meglumine and diatrizoate sodium, 76% for injection; E. R. Squibb & Sons, Princeton, N.J.) in 0.01 M HEPES containing 0.15 M NaCl and centrifuged at 43,000 × *g* for 1 h at 4°C by using a rotor (SW27; Beckman Instruments, Inc., Fullerton, Calif.), as described by Caldwell et al. (5).

Sodium dodecyl sulfate-PAGE. Partially purified chlamydial EBs were treated with solubilizing solution and boiled for 10 min as described by Caldwell et al. (5). The polypeptides were separated on a 12.5% sodium dodecyl sulfate-polyacrylamide discontinuous gel by the technique introduced by Laemmli (11). The resolved chlamydial proteins were electrophoretically transferred onto a nitrocellulose membrane (NCM) as described by Towbin et al. (24). For preliminary testing, such as screening of anti-chlamydial sera, single strips cut from a large blot were used. The large blot was prepared by using a single-well comb (PROTEAN II; Bio-Rad Laboratories, Richmond, Calif.) in which a 5-mm-wide well was cut at one end for the markers and the rest of the comb was used for electrophoresis of chlamydial proteins. A total of 1 mg of protein in 2 ml of chlamydial suspension and solubilizing solution (1:1) was applied to the whole gel. Alternatively, the principal immunoblotting assays were run on the blots prepared from either 15- or 25-well combs (PROTEAN II; Bio-Rad).

Immunoblot technique. NCM blots were first stained with Ponceau S to make sure that the chlamydial proteins were transferred successfully. The blots were blocked with Blotto (10) for 1 h, were exposed to a 1:40 dilution of antiserum in diluent (10% goat serum in phosphate-buffered saline), and rocked on a platform at room temperature overnight. The blots were washed with phosphate-buffered saline four times. The blots were then exposed to the appropriate horseradish peroxidase-conjugated anti-immunoglobulins (1:500 dilution of the antiserum for 2 h). The antibody-binding reactions on the blots were detected by the addition of substrate (hydrogen peroxide–4-chloro-1-naphthol; Sigma). Alternatively, the first step in the reaction was followed by washing and the addition of biotin-labeled anti-immunoglobulins (1:500 in diluent for 2 h). The blots were washed and were reacted with horseradish peroxidase-la-

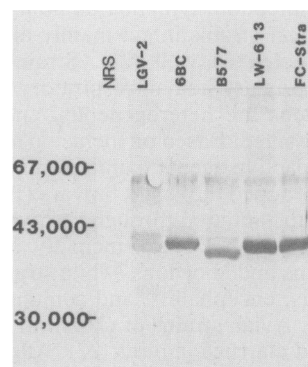

FIG. 1. Proteins of chlamydial EBs from different *C. psittaci* strains and a *C. trachomatis* (LGV-2) strain were resolved by sodium dodecyl sulfate-PAGE and transferred electrophoretically onto NCMs. The blot was exposed to a 1:40 dilution of a PMA from rabbit 2ML that was immunized with the MOMP of a serovar 2 strain (LW-613) of *C. psittaci*. The reactions between MOMPs and antisera were detected with horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G followed by the addition of substrate. B577 is a serovar 1 strain; LW-613 and FC-Stra are serovar 2 strains, 6BC is an avian strain of *C. psittaci*, and LGV-2 is a strain of *C. trachomatis*. Normal rabbit serum (NRS) was reacted with LW-613. Numbers on the left are molecular weights.

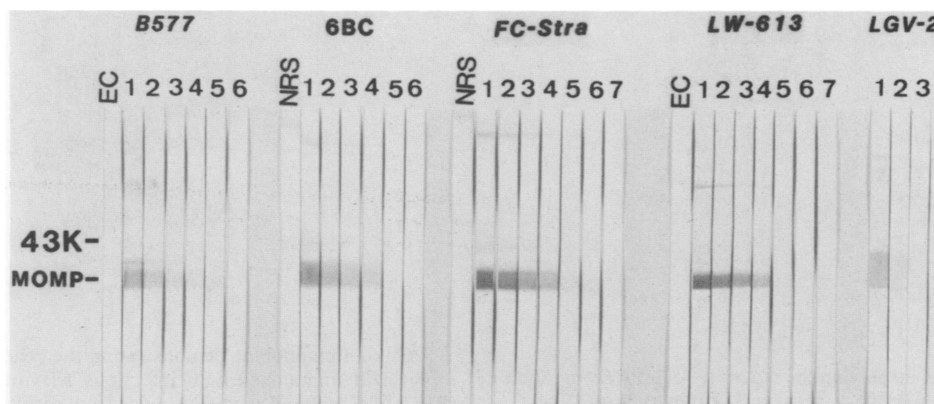

FIG. 2. Immunoblot demonstrating the reaction between blots from different chlamydial strains with twofold serially diluted serum from rabbit 2ML, as described in the legend to Fig. 1. 43K, 43,000 molecular weight; EC, enzyme conjugate control; NRS, normal rabbit serum.

beled-streptavidin (KPL; 1:1,000 in diluent for 1 h) followed by exposure to the substrate.

Preparation of antisera and MAbs. PMAs against MOMPs of different serovars of *C. psittaci* were produced in rabbits by using antigen that was prepared by three different methods. First, rabbits were immunized subcutaneously and intraperitoneally with the MOMP that was cut out from a Coomassie brilliant blue-stained polyacrylamide gel and crushed in phosphate-buffered saline. Second, the MOMP was transferred to NCM as a large blot, and it was stained with Ponceau S to localize the MOMP. The MOMP on the NCM was cut out and dissolved in dimethyl sulfoxide (Sigma) and precipitated in bicarbonate buffer as described previously (1). The precipitated antigen was used to boost the animal subcutaneously and intraperitoneally 5 months after the initial inoculation. The third boost was induced by subcutaneously and intraperitoneally implanting the MOMP-containing NCM (21) 2 months following the second inoculation. Blood was collected for serum harvest 4 weeks after this boost.

MAbs against the MOMP of serovar 2 (FC-Stra) were made by a previously described technique (7) by using intraperitoneal implantations of MOMP-containing NCMs (21). PAs were available from rabbits that were immunized with single subcutaneous inoculations of infectious, yolk sac-propagated EBs that were partially purified.

RESULTS

Immunoblot reactions of anti-MOMP PMAs with MOMPs of different chlamydial strains. Sera from rabbit 1MB, which was immunized with a MOMP of a serovar strain 1 (B577), and rabbit 2ML, which was immunized with a MOMP of a serovar 2 strain (LW-613), reacted with MOMPs of all strains of *C. psittaci* at dilutions of 1:1,280 and 1:2,560, while they reacted at a dilution of 1:20 or 1:40 with the strain LGV-2 MOMP, as summarized in Table 1 and illustrated in Fig. 1 and 2, for which serum from rabbit 2ML was used. Notice the difference in the molecular masses of MOMPs from strains B577 and 6BC. Serum from rabbit 9MF, which was immunized with a serovar 2 strain (FC-Stra), reacted with MOMPs of the *C. psittaci* strains at dilutions of 1:2,560 and 1:5,120, but it did not detect the LGV-2 MOMP at a 1:10 dilution (Table 1).

Immunoblot reactions of MAb against the serovar 2 MOMP with MOMPs of other chlamydial strains. The MAb against the serovar 2 MOMP reacted with MOMPs of all *C. psittaci* strains tested, but it failed to react with the LGV-2 MOMP (Table 1 and Fig. 3).

Immunoblot reactions of PAs produced against infectious EBs. Sera from five rabbits, all of which were immunized with infectious EBs of a serovar 2 strain, reacted specifically with the MOMPs of the homologous strains and did not recognize MOMPs of any other strains of *C. psittaci* or LGV-2 (Table 1 and Fig. 4). One of the PAs that was produced against serovar 1 EBs recognized the MOMP of the homologous strain and that of an avian strain (6BC). It did not react with the MOMPs of any other *C. psittaci* strains or with the MOMP of the LGV-2 strain (Table 1 and Fig. 5). The other three PAs against the serovar 1 strain reacted with the MOMPs of homologous strains only (Table 1 and Fig. 6).

DISCUSSION

We produced an MAb, PMAs, and PAs against reduced PAGE-separated MOMPs or against infectious EBs of *C. psittaci* strains. The reactivities of these antisera against MOMPs of different *C. psittaci* strains and a strain of *C. trachomatis*, LGV-2, were examined in an immunoblot assay. An MAb and a PMA produced against a serovar 2 strain MOMP reacted strongly with MOMPs of *C. psittaci* strains but did not react with the MOMP of LGV-2 (Table 1

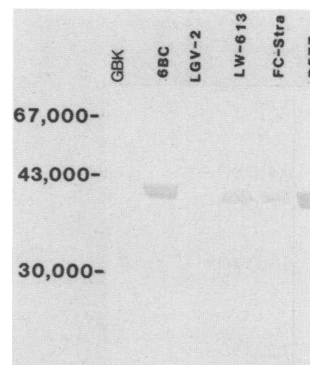

FIG. 3. Immunoblots demonstrating the reaction of a MAb raised against a serovar 2 strain (FC-Stra) of *C. psittaci*. The reactions between the supernatants of the cloned cells and chlamydial MOMPs were detected by using horseradish peroxidase-conjugated goat anti-mouse immunoglobulins followed by the addition of substrate. Details of the immunoblotting steps are given in the legend to Fig. 1. Numbers on the left are molecular weights.

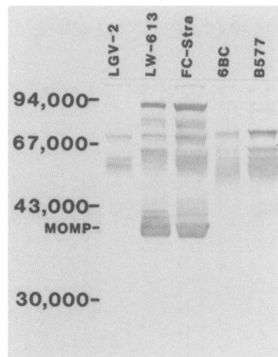

FIG. 4. Immunoblot displaying the reactivity of a PA from rabbit 2PL immunized with EBs of a serovar 2 strain (LW-613) of *C. psittaci* against proteins from different strains of chlamydiae. The blot was exposed to an antiserum dilution of 1:40. Reactions between antiserum and chlamydial proteins were detected by the procedure described in the legend to Fig. 1. Numbers on the left are molecular weights.

and Fig. 3 and 4). The reactions revealed that these antibodies recognized species-specific epitopes on the MOMPs of *C. psittaci* strains but not those of the LGV-2 strain of *C. trachomatis*. The reactions with the MOMP of *C. pneumoniae* need to be analyzed. PMAs produced against MOMPs of serovar 1 or serovar 2 strains of *C. psittaci* reacted at dilutions of 1:1,280 to 1:2,560 with MOMPs of all *C. psittaci* strains, and they had a titer of 20 or 40 with the MOMP of the LGV-2 strain (Table 1 and Fig. 1 and 2). Evidently, these two antisera recognized both genus- and species-specific epitopes on chlamydial MOMPs, confirming the findings of Mondesire and co-workers (12).

Five PAs against infectious LW-613 EBs of serovar 2 that were produced in rabbits only reacted with MOMPs of the homologous serovars and did not recognize the MOMPs of any other *C. psittaci* strains or that of strain LGV-2 (Table 1 and Fig. 4). Four PAs were produced against EBs of a serovar 1 strain (B577). Three of these PAs reacted with MOMPs of homologous strains and failed to recognize the MOMPs of other chlamydial strains (Table 1 and Fig. 6). The other PA reacted with MOMPs of homologous strains as well

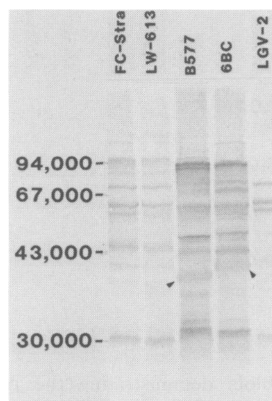

FIG. 5. Immunoblot demonstrating the reaction of polyclonal serum from rabbit 3PB immunized with infectious EBs of a serovar 1 strain (B577) of *C. psittaci* against proteins from different strains of chlamydiae. Details are given in the legend to Fig. 1. Numbers on the left are molecular weights.

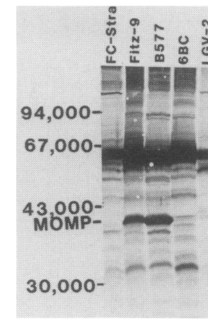

FIG. 6. Immunoblot demonstrating the reaction of serum from rabbit 1PB immunized with infectious EBs of a serovar 1 strain (B577) of *C. psittaci* against proteins from serovar 1 strains B577 and Fitz-9 and other chlamydial strains. Details are given in the text on the biotin-streptavidin system used. Numbers on the left are molecular weights.

as with that of the avian strain 6BC, but it did not react with the MOMPs of other strains of *C. psittaci* or that of LGV-2 (Table 1 and Fig. 5). The observed cross-reaction with the avian strain was not due to any cross contamination of the two chlamydial strains, because the other antiserum samples against strain B577 reacted only with the MOMP of B577 from the same antigen preparation. Furthermore, the molecular masses of the MOMPs of the two strains differed significantly enough to exclude this possibility. These results are in agreement with the observations by other investigators who reported the presence of genus-, species-, and serovar-specific epitopes on the MOMPs of *C. trachomatis* and *C. psittaci* (2, 3, 5, 6, 12, 14, 20, 29).

The antibody responses to infectious EBs are principally against the serovar-specific epitopes of the MOMPs. The immunogenic variable domains of chlamydial MOMPs with serovar specificities are surface-exposed epitopes on the intact EBs (29). Thus, they are readily accessible to the immune system. MOMP is a cystein-rich macromolecule with disulfide-linked complexes (4, 15). Separation and reduction of MOMPs by PAGE changes the conformational epitopes that are associated with variable domains, leading to the exposure of conserved MOMP sequences. This explains why reduced PAGE-separated MOMP antigens stimulated mainly genus- or species-specific antibodies. Furthermore, the PMAs produced against reduced PAGE-separated MOMPs did not neutralize the infectivity for cultured cells (unpublished data). Consequently, we do not consider it advisable to use reduced PAGE-separated antigens for antibody production in the study of candidate antigens for vaccine development. Serovar-specific reactions that were observed with rabbit polyclonal antisera indicate that infectious EBs evoke specific responses that are probably important for limiting the infection. Chlamydial MOMP is involved in structural integrity, pathogenicity, as well as the neutralization of infectivity for cultured cells and animal models (2, 4, 16, 29). These properties identify this protein as an important antigen in designing potential vaccines.

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