A Chlamydia trachomatis OmcB C-Terminal Fragment Is Released into the Host Cell Cytosol and Is Immunogenic in Humans

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The Chlamydia trachomatis outer membrane complex protein B (OmcB) is an antigen with diagnostic and vaccine relevance. To further characterize OmcB, we generated antibodies against OmcB C-terminal (OmcBc) and N-terminal (OmcBn) fragments. Surprisingly, the anti-OmcBc antibody detected dominant signals in the host cell cytosol, while the anti-OmcBn antibody exclusively labeled intra-inclusion signals in C. trachomatis-infected cells permeabilized with saponin. Western blot analyses revealed that OmcB was partially processed into OmcBc and OmcBn fragments. The processed OmcBc was released into host cell cytosol, while the OmcBn and remaining full-length OmcB were retained within the chlamydial inclusions. The organism-associated OmcB epitopes became detectable only after the C. trachomatis-infected cells were permeabilized with strong detergents such as SDS. However, the harsh permeabilization conditions also led to the leakage of the already secreted OmcBc and chlamydia-secreted protease (CPAF) out of the host cells. The OmcBc processing and release occurred in all biovars of C. trachomatis. Moreover, the released OmcBc but not the retained OmcBn was highly immunogenic in C. trachomatis-infected women, which is consistent with the concept that exposure of chlamydial proteins to host cell cytosol is accompanied by increased immunogenicity. These observations have provided important information for further exploring/optimizing OmcB as a target for the development of diagnostic methods and vaccines.

Chlamydia trachomatis is the most frequently reported bacterial sexually transmitted disease in the United States. In 2008, 1.2 million chlamydial infections were reported to the CDC (6). If untreated, more than 10% of C. trachomatis-infected women may develop complications such as pelvic inflammatory diseases, ectopic pregnancy, and infertility (34). The annual cost of treating C. trachomatis complications in women is >$2 billion (12, 51). Due to the lack of obvious symptoms after an acute infection, most infected individuals do not seek treatment, thus permitting the development of complications. One solution to this challenge is rapid diagnosis so that antibiotics can be taken to reduce complications. The current diagnosis of chlamydial infection is based on nucleic acid detection, requiring specialized labs and taking days for health care providers or patients to obtain results. Attempts to immunobiochemically detect the most abundant major outer membrane protein (MOMP) as a rapid diagnosis method have been unsuccessful due to the difficulty in solubilizing MOMP. A second (or long-term) solution is vaccination so that exposure to C. trachomatis no longer causes complications. The failure of whole-organism-based vaccines more than 50 years ago (26, 27) and immunological studies since then (42–44) have led to the conclusion that a subunit chlamydial vaccine is both necessary and feasible (52). However, there is still no licensed C. trachomatis vaccine.

The chlamydial outer membrane complex protein B (OmcB) is the second most abundant outer membrane protein; it contains 24 cysteine residues and has a molecular mass of 60 kDa and thus is also called the cysteine-rich 60-kDa protein (1, 48). OmcB is highly conserved among Chlamydia species (21), suggesting that it plays a significant role during intracellular chlamydial infection. OmcB may function as an adhesin for chlamydial invasion into host cells (17, 18), since heparin can block the infectivity of some C. trachomatis serovars by binding to an N-terminal peptide of OmcB (41, 56). The internalized elementary body (EB) can then differentiate into a noninfectious but metabolically active reticulate body (RB) that starts biosynthesis and undergoes replication. The progeny RBs differentiate back into EBs for spreading to nearby cells. OmcB is involved in the conversion of RBs to EBs (45, 48) and is thought to contribute to the cell wall rigidity and osmotic stability of the EB (48). During the chlamydial intracellular growth cycle, which takes 48 to 72 h to complete in vitro, the organisms secrete numerous proteins into host cells (5, 58, 64, 66).

OmcB has been recognized as an immunodominant antigen during chlamydial infection, inducing robust immune responses in both humans (23, 25, 46, 60) and animals (53, 59, 62). Due to its abundance and strong immunogenicity, OmcB has been considered a target for developing both serodiagnosis methods (2, 21) and subunit vaccines (15, 49, 50). Nevertheless, there has been considerable debate regarding the precise location and role of OmcB during C. trachomatis infection. OmcB was reported to localize at the inner surface of the outer membrane and to become surface accessible only after treatment with reducing reagents and proteases (45). The immunodominant regions of OmcB have not been mapped. Various

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heparin blockade studies (11, 41, 56, 63) suggest that the N-terminal region of OmcB is surface exposed. The identification of CD8 epitopes at the OmcB C terminus (23) suggests that the C-terminal region is accessible to the host cell cytosol. Since exposure of chlamydial proteins to host cell cytosol often correlates with increased immunogenicity (35, 60), we hypothesize that the OmcB C-terminal region may be immunodominant. Clearly, further characterization of OmcB is still necessary. In the current study, we report that OmcB is partially processed into C-terminal (OmcBc) and N-terminal (OmcBn) fragments and that the processed OmcBc is released into the host cell cytosol while the processed OmcBn and remaining full-length OmcB are retained within the chlamydial inclusions. Interestingly, it is the released OmcB (but not the retained OmcBn) that is highly immunogenic during chlamydial infection in humans. The finding of the release of OmcB to host cell cytosol not only provides a molecular explanation for the immunodominance of the OmcB C-terminal region but also suggests that the outer membrane protein OmcB can participate in chlamydial intracellular interactions with host cells.

MATERIALS AND METHODS

Cell culture and chlamydial infection. HeLa cells (human cervical carcinoma epithelial cells; ATCC CCL2), Chlamydia muridarum (ATCC CCL2), and C. trachomatis Sc-24; Santa Cruz Biotechnology, CA). In addition, human antisera pooled from 8 healthy women or 20 women urogenitally infected with C. trachomatis serovar D genome into pgEX vectors (Amersham Pharmacia Biotech, Inc., Piscataway, NJ). The following primers were used: full-length OmcB (covering the codon for amino acid 451 to the codon for amino acid 553) forward primer 5′-CGC(spacer)-GGATCC(restriction site)-ATGTCTACAAACGTTATTAGCTTAG-3′ and back primer 5′-TTTTCCTTTT-GCGGCCGC-TTAATTTGTGAATTGTCCAGGAGTT-3′, and back primer 5′-CGC-GGATC-ATGACGTTTACTCTTGGAGATATG-3′ and back primer 5′-TTTTCCTTTT-GCGGCCGC-TTACAGTACACGCTGTCCAGA-3′ and back primer 5′-TTTTCCTTTT-GCGGCCGC-TTACAGTACACGC

The chlamydial organisms were either purchased from ATCC (Manassas, VA) or acquired from Harlan Caldwell at the Rocky Mountain Laboratory, NIAID/NH, Hamilton, Montana (28), or anti-OmcB (from Harlan Caldwell, NH, or Thomas Hatch, University of Tennessee; designated Caldwell and Hatch rabbit antibodies, respectively) plus a goat anti-rabbit IgG secondary antibody conjugated with Cy3 (green; Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) was used to visualize chlamydial organisms, inclusion membrane, or OmcB. The various mouse antibodies plus a goat anti-mouse IgG conjugated with Cy3 (red; Jackson ImmunoResearch) were used to visualize the corresponding antigens. The mouse antibodies included polyclonal antibodies (PAs) made against GST-OmcBc and GST-OmcBn (both from the current study) and CTB13 (a known IgG) (17) fusion proteins and monoclonal antibodies (MAbs) 100a against CPAF (65) and MC22 against the major outer membrane protein (MOMP) (65). In some cases, the primary antibodies were preabsorbed with either the corresponding or heterologous agaroase bead-immobilized fusion proteins prior to immunostaining. The immunofluorescence images were acquired and processed as described previously (19).

Western blot assay. The Western blot assay was carried out as described elsewhere (67, 68). For monitoring SDS-induced protein release, HeLa cells infected with Chlamydia trachomatis organisms were fixed with 2% paraformaldehyde (Sigma, St. Louis, MO) dissolved in phosphate-buffered saline (PBS) for 1 hr at room temperature, followed by permeabilization with 2% saponin (Sigma) for at least 1 h. In some experiments, cell samples were blocked, the cell samples were subjected to antibody and chemical staining. Hoechst stain (blue; Sigma) was used to visualize DNA. A rabbit anti-chlamydial outer membrane protein antibody (RIL2, referred to also as RIL21; published data), anti-InCA (kindly provided by Ted Hackstadt, Laboratory of Intracellular Parasites, Rocky Mountain Laboratories, NIAID, NIH, Hamilton, Montana) (28), or anti-OmcB (from Harlan Caldwell, NH, or Thomas Hatch, University of Tennessee; designated Caldwell and Hatch rabbit antibodies, respectively) plus a goat anti-rabbit IgG secondary antibody conjugated with Cy2 (green; Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) was used to visualize the various chlamydial inclusions, inclusion membrane, or OmcB. The various mouse antibodies plus a goat anti-mouse IgG conjugated with Cy3 (red; Jackson ImmunoResearch) were used to visualize the corresponding antigens. The mouse antibodies included polyclonal antibodies (PAs) made against GST-OmcBc and GST-OmcBn (both from the current study) and CTB13 (a known IgG) (17) fusion proteins and monoclonal antibodies (MAbs) 100a against CPAF (65) and MC22 against the major outer membrane protein (MOMP) (65). In some cases, the primary antibodies were preabsorbed with either the corresponding or heterologous agaroase bead-immobilized fusion proteins prior to immunostaining. The immunofluorescence images were acquired and processed as described previously (19).
were also used, which were approved by the IRB under an exemption 4 protocol. These human serum samples were left over from previous studies (54), and no patient information can be traced from these samples.

**Fusion protein microplate ELISA.** The enzyme-linked immunosorbent assay (ELISA) was carried out as described previously (55). Briefly, the GST fusion protein-containing bacterial lysates were added to glutathione-coated 96-well microplates (catalog no. 15140B; Pierce, Rockford, IL), and after blocking with 2.5% (wt/vol) milk dissolved in PBS, the plates were used to assay antibody reactivity. All primary antibodies were preabsorbed with a bacterial lysate containing GST alone before they were assayed on the ELISA plates. The human protein-containing bacterial lysates were added to glutathione-coated 96-well microplates (catalog no. 15140B; Pierce, Rockford, IL), and after blocking with 2.5% (wt/vol) milk dissolved in PBS, the plates were used to assay antibody reactivity. All primary antibodies were preabsorbed with a bacterial lysate containing GST alone before they were assayed on the ELISA plates.

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**FIG. 1.** Detection of CT443c in the cytosol of *C. trachomatis*-infected cells. HeLa cells infected with *C. trachomatis* were processed for immunolabeling with mouse anti-OmcB antibodies (as indicated on the left) and visualized with a goat anti-mouse IgG conjugated with Cy3, a rabbit anti-chlamydial organism antibody visualized with a Cy2-conjugated goat anti-rabbit IgG, and the DNA dye Hoechst. The mouse antibodies raised with GST-OmcB C terminus (OmcBc) (a to d) and GST-OmcB N terminus (OmcBn) (e to h) fusion proteins were used at various dilutions as indicated at the top. Note that as dilution of the mouse antisera increased, cytosolic signals labeled with anti-OmcBc became clear, while the anti-OmcBn signal was always restricted within the inclusions. Red arrow indicate OmcBc secreted into host cell cytosol.

**FIG. 2.** Specificity of the anti-GST–OmcBc and –OmcBn fusion protein antibodies. Tricolor immunofluorescence labeling was carried out as described in the Fig. 1 legend except that the mouse antibodies as displayed on the left were preabsorbed with or without corresponding or control GST fusion proteins as displayed at the top prior to the detection of the endogenous proteins in *C. trachomatis*-infected cells. Note that the signals detected by the anti-OmcBc and anti-CPAF antibodies (in the cytosol of the infected cells [a and i]) and the anti-OmcBn antibody (in chlamydial inclusions [e]) were removed by preabsorption with the corresponding (h, l, and g) but not control (c and d, j and k, and f and h, respectively) fusion proteins. The chlamydial organism and DNA labelings remained constant regardless of the mouse antibody absorption.
and mouse antisera were obtained and produced as described above and previously (55, 60). Goat anti-human or -mouse IgG secondary antibodies conjugated with HRP (catalog no. 109-035-064 and 711-035-152, respectively; Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) were used to probe the primary antibody binding. The soluble substrate ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt] (catalog no. A1888-5G; Sigma) was used to visualize the reactions, and the reactivity was recorded as absorbance (optical density [OD] at 405 nm). A bacterial lysate-coated well with GST alone in each plate was used as a negative control, and the OD value of the GST well was controlled at 0.05 or lower. Any wells with an OD value equal to or greater than 4 times of the OD value from the GST well were considered positive.

RESULTS

Detection of OmcB in the cytosol of C. trachomatis-infected cells by antibodies against C- but not N-terminal fragments of OmcB. Antibodies raised against OmcB C-terminal (covering amino acids T270 to Y553) and N-terminal (S41 to G165) fragments were used to localize the endogenous OmcB in C. trachomatis-infected cells in a standard immunofluorescence assay (Fig. 1). The antibody against the OmcB C-terminal fragment (OmcBc) labeled intensive signals in the cytoplasm of most infected cells, while the antibody against the OmcB N-terminal fragment (OmcBn) detected only signals inside the chlamydial inclusions, which is inconsistent with the concept that OmcB is a major component of the chlamydial outer membrane complex (3, 16, 38, 40, 56). The antibody specificity was confirmed using an absorption approach (Fig. 2). The cytosolic staining of OmcBc was removed by absorption with GST-OmcBc but not by absorption with GST-OmcBn or the control CPAF (a chlamydia-secreted protease [65]). Conversely, the inclusion labeling of OmcBn was blocked only by absorption with GST-OmcBn and not by that with other fusion proteins. As a control, the CPAF labeling was blocked only by absorption with the GST-CPAF fusion protein. These results have demonstrated that the anti-OmcBc and -OmcBn antibodies specifically detected the corresponding endogenous epitopes without cross-reacting with each other or CPAF. The question is how the two antibodies recognizing different re-

FIG. 3. Permeabilization with SDS increases the accessibility of chlamydial organism-associated OmcB to antibody labeling. Tricolor immunofluorescence labeling was carried out as described in the Fig. 1 legend except that HeLa cells infected with C. trachomatis organisms were differentially permeabilized. As listed on the left, the paraformaldehyde-fixed HeLa cell samples were permeabilized with 2% saponin for 1 h as usual (standard protocol) (a to d) or with 1% SDS for 3 min (e to h) or 40 min (i to l). (A) Various mouse antibodies as displayed at the top were used to label corresponding antigens (red), along with a rabbit antibody to visualize the chlamydial organisms (green) and Hoechst DNA dye to visualize nuclei (blue). (B) The SDS-enhanced colocalization of anti-OmcB and anti-MOMP labelings with chlamydial organisms was confirmed using confocal microscopy. Note that as the SDS treatment was prolonged, the anti-OmcB (k and t), anti-OmcBn (n and w), and anti-MOMP (q and z) antibodies labeled more signals (red) that overlapped with the chlamydial organisms (green). (C) The parallel chlamydia-infected cell samples were also fixed with cold methanol for 10 min (d to f) prior to the immunofluorescence labeling. The mouse anti-OmcBc (a and d) and two rabbit anti-OmcB polyclonal antibodies (designated Caldwell and Hatch) (b, e, c, and f) labeled signals in the host cell cytosol when cells were treated with paraformaldehyde (PF) and saponin (a, b, and c) (red or green arrows) but only inside the inclusions when cells were treated with methanol (d, e, and f).
gions of the same OmcB molecules detected signals in different subcellular locations.

**Permeabilization of *C. trachomatis*-infected cells with SDS enables both anti-OmcBc and anti-OmcBn antibodies to detect chlamydial organism-associated OmcB epitopes.** The distinct distribution patterns of anti-OmcBc and anti-OmcBn antibody labelings under the mild permeabilization conditions described above may be due to differential accessibility of epitopes in the two different regions of OmcB that are known to tightly associate with the outer membrane complex (9, 38). Permeabilization with 1% SDS was used to increase the accessibility of the OmcB epitopes (Fig. 3). As the SDS treatment was prolonged, both the anti-OmcBc and anti-OmcBn antibodies labeled more signals that overlapped with the chlamydial organisms (Fig. 3A), which was confirmed using confocal microscopy (Fig. 3B), indicating that both anti-OmcB antibodies can recognize OmcB epitopes associated with chlamydial organisms. At the same time, the SDS treatment also resulted in loss of the cytosolic signals labeled by anti-OmcBc and anti-CPAF antibodies, suggesting that the harsh SDS treatment may cause leakage of the chlamydial signals already in host cell cytosol out of the host cells. We also monitored the OmcB intracellular distribution in methanol-fixed cells (Fig. 3C). After methanol fixation, the mouse anti-OmcBc from the current study and two rabbit anti-OmcB antibodies obtained from H. Caldwell and T. Hatch, respectively (these rabbit antibodies were raised with full-length OmcB), labeled strong inclusions signals without any significant signals in the host cell cytosol. However, these same antibodies detected overwhelming signals in the host cell cytosol when the infected cells were fixed with paraformaldehyde and permeabilized with saponin. These results together suggest that a portion of the OmcB molecules may be processed, the processed fragment recognized by the anti-OmcBc but not the anti-OmcBn antibodies is released into host cell cytosol, and the cytosolic OmcBc can further leak out of the host cells under SDS treatment.

**OmcB is partially processed into OmcBc and OmcBn fragments, and the processed OmcBc fragment is released into the host cell cytosol.** To test whether OmcB is processed and the processed fragment leaks out of host cells upon SDS treatment, the supernatants and cell pellets from the *C. trachomatis*-infected cultures were harvested separately for monitoring the fate of OmcB and its putative fragments in a Western blot assay (Fig. 4). When the infected cells were permeabilized with saponin, all signals were retained in the cell pellet. The control anti-CPAF MAb 100a detected the 35-kDa CPAF C-terminal fragment that is generated by CPAF autoprocessing (13, 14, 29, 65). Besides the full-length OmcB and various incomplete reduction or degradation species, the anti-OmcBc antibody detected a short fragment migrating at ~40 kDa, while the anti-OmcBn antibody detected a fragment migrating at ~20 kDa, suggesting that OmcB was partially processed into a 40-kDa C-terminal fragment (OmcBc) and a 20-kDa N-terminal fragment (OmcBn). When the infected cells were permeabilized with SDS, both CPAF and OmcBc leaked into the supernatants, while OmcBn and full-length OmcB remained in the cell samples regardless of how long the cells were treated with SDS. These observations not only provide a molecular explanation for the observed loss of anti-OmcBc and CPAF signals from SDS-treated cell samples under immunofluorescence microscopy but also demonstrate that OmcB is processed and the processed OmcBc may be responsible for the secreted signals detected by the anti-OmcBc antibody.

To directly visualize the structural basis of the cytosolic signals detected by the anti-OmcBc antibody, the infected cells were fractionated into nuclear (Ct-HeLa pellet, containing chlamydial inclusions) (lane 3) and cytosolic (Ct-HeLa S100, containing chlamydia-secreted proteins) (lane 4) fractions. The cellular fractions along with total cell lysates (normal HeLa [lane 1] and Ct-HeLa [lane 2]) and purified chlamydial RB (lane 5) and EB (lane 6) organisms were resolved in an SDS-polyacrylamide gel for Western blot detection with antibodies against OmcBc (a), OmcBn (b), CPAFc (CPAF C-terminal fragment of 35 kDa, processed during chlamydial infection and recognized by MAb 100a) (c), CT813 (an inclusion membrane protein) (d), MOMP (e), and human HSP70 (f). Note that all antibodies detected their corresponding antigens in the Ct-HeLa whole-cell lysate and other corresponding samples (as indicated on the right). The OmcBc was highly enriched in the cytosolic fraction, while both the full-length OmcBc and OmcBc fragment always associated with the chlamydial organisms or Ct-HeLa pellet fraction.
pellet fraction, indicating no contamination of the pellet fraction by cytosolic components. As expected, the control secretion protein CPAF was detected only in the S100 fraction and not in the pellet fraction. Like CPAF, most of the OmcBc fragments were detected in the S100 fraction, while unlike CPAF, most of the OmcBn fragments were detected in the pellet. These results demonstrated that the processed OmcBc but not OmcBn was released into the host cell cytosol.

Expression and distribution of OmcBc during *C. trachomatis* infection. To monitor the full-length OmcB protein expression, infected cells were permeabilized with 1% SDS (Fig. 6). The preexisting OmcB in the EBs was detected at 2 h but disappeared at 12 h and 18 h after infection. The newly synthesized OmcBc signal was obvious in the central region of the inclusion by 24 h (d). CPAF was detected at 18 h postinfection. With 1% SDS permeabilization, the preexisting OmcBc was detected as early as 2 h (k) but disappeared up to 18 h (l and m). The newly synthesized OmcBc signal was obvious in the central region of the inclusion by 24 h (n). MOMP was detected throughout the growth cycle and associated with the organisms (from 2 h to 30 h) (p to t). Red arrows in the insets indicate the first detection of the corresponding antigens, while those in the main panels indicate the first detection of secreted proteins.
K), lymphogranuloma venereum (LGV) biovar (L1 to -3), or murine biovar (MoPn) organisms were processed at 28 h (for MoPn) or 40 h after infection for immunofluorescence labeling (Fig. 7). The anti-OmcBc antibody detected significant signals in the cytosol of host cells infected with all biovars.

The released OmcBc but not the retained OmcBn is highly immunogenic during chlamydial infection in humans. Because chlamydial proteins exposed to host cell cytosol are commonly highly antigenic (35, 60), we hypothesized that OmcBc is more antigenic than OmcBn. Since OmcB is highly immunogenic during C. trachomatis infection in humans (23, 60, 61), we used 20 antisera from women urogenitally infected with C. trachomatis to map the immunodominant regions of OmcB by both ELISA (Fig. 8) and Western blotting (Fig. 9). Although these 20 women might be infected with different serovars, their antibodies should be able to recognize OmcB peptides from serovar D because OmcB is highly conserved among all C. trachomatis serovars (http://stdgen.northwestern.edu). In ELISA, all 20 antisera positively recognized the full-length OmcB and the control CPAF and Pgp3 fusion proteins (Fig. 8a). Interestingly, most or all of the 20 human antisera recognized fragments from the OmcB C terminus, while only one human antisera recognized the N-terminal fragments. When the 20 human antisera were pooled, similar results were obtained (Fig. 8c). The human antibody reactivity with the C-terminal fragments was specific, since the reactivity was removed by absorption with lysates made from C. trachomatis-infected (Fig. 8e) but not normal (Fig. 8d) HeLa cells. A pooled negative human antisera sample failed to react with any GST fusion proteins. A mouse antiserum raised against a recombinant OmcB fusion protein reacted with most OmcB fragments. The dominant recognition of OmcB C-terminal fragments by human antibodies was also confirmed using a Western blot assay (Fig. 9). The sample of 20 pooled human antisera recognized both OmcB and CPAF but not Pgp3 on Western blotting, which is consistent with previous observations that disruption of the Pgp3 trimers can completely block human antibody recognition (9, 37). Interestingly, the pooled positive antiserum recognized all C-terminal fragments of OmcB but no N-terminal fragments even when the dilution of the human antisera was low, confirming the immunodominance of the OmcB C terminus. As the dilution of the human antisera increased, the F12 fragment (covering residues 411 to 553) but not other fragments exhibited reactivity similar to that of the full-length OmcB, suggesting that the very C-terminal region of OmcB might account for most of the immunogenicity of OmcB in humans.

**DISCUSSION**

OmcB is a highly conserved and abundant protein that is associated with the chlamydial outer membrane complex. Due to its abundance and immunogenicity during chlamydial infection in humans (23, 25, 46, 60), OmcB has been considered a target for both diagnostics and vaccine development (2, 15, 21, 49, 50). In the current study, we have found that OmcB is
partially processed into OmcBc and OmcBn fragments and that the processed OmcBc is released into host cell cytosol. These are important and biologically relevant findings. First, the anti-OmcBc antibody detected overwhelming signals in the cytosol of *C. trachomatis*-infected cells permeabilized with saponin for 1 h, a treatment condition that is able to selectively permeabilize the lipid membrane by removing membrane cholesterol but without altering the integrity of membrane protein complexes (30, 31). Under such a permeabilization condition, the anti-OmcBc antibody was able to access the epitopes of the

FIG. 8. Mapping immunodominant regions of OmcB recognized by human antibodies in ELISA. The full-length (FL) OmcB and its fragments, including F1 to F12, along with various other control proteins were expressed as GST fusion proteins (as displayed along the x axis at the bottom). The GST fusion proteins were applied to glutathione-conjugated microplates for reacting with human (a to f) or mouse (g) antibodies. A goat anti-human or mouse IgG conjugate was used to detect human or mouse antibody binding. (a) Reactivity of each GST fusion protein with each of the 20 antisera from *C. trachomatis*-infected women. All human antisera were used at a final dilution of 1:500. A positive reactivity between a given GST fusion protein and an antiserum sample was defined as an OD value equal to or greater than 4 times the OD value obtained from the well coated with GST alone in the same microplate. Each positive reaction was represented with a horizontal bar. (b) Average OD value for each GST fusion protein based on its reactivity with the 20 antisera shown in panel a. (c to e) The 20 human antisera were further pooled at an equal ratio, and the pooled antiserum (designated pooled positive [+ve] antiserum) reacted with the GST fusion proteins without absorption (c) or with absorption with HeLa lysates alone (d) or *C. trachomatis*-infected HeLa (e) lysates. (f) A pooled antiserum sample from eight healthy individuals without chlamydial infection (designated pooled negative [-ve] antiserum) was similarly reacted with the GST fusion proteins. All pooled human serum samples were used at a final dilution of 1:200. (g) A mouse antiserum raised with a full-length OmcB protein purified by cleaving OmcB from the GST-OmcB fusion protein immobilized on glutathione-agarose beads was also reacted with the GST fusion proteins at a dilution of 1:500. Note that as stated in Materials and Methods, all human and mouse antiserum samples were preabsorbed with bacterial lysate containing GST alone prior to any treatments or reaction with the GST fusion proteins. Although the positive human antibodies reacted only with the C-terminal fragments of OmcB, the mouse antiserum reacted with most OmcB fragments.

FIG. 9. The C-terminal fragments of OmcB are dominantly recognized by human antibodies. (a) GST-OmcB fragments and other GST fusion proteins as described in the Fig. 8 legend were resolved in an SDS-polyacrylamide gel, and the resolved protein bands were blotted onto a nitrocellulose membrane for reacting with pooled positive (+ve) or negative (-ve) human antiserum as described in the Fig. 8 legend. (b to e) The positive antiserum were used at final concentrations of 1:10,000 (b), 1:50,000 (c), and 1:250,000 (d), while the negative antiserum were used at 1:10,000 (e). Note that the positive human antibody reacted only with various C-terminal fragments of OmcB, and the reactivity with F12 (covering residues 411 to 533, marked with # in panel d) was much higher than that with CPAF (marked with $ in panel d), a known immunodominant antigen of *C. trachomatis*, and could account for most of the immunogenicity of OmcB (marked with * in panel d) in humans. Pgp3 is a known conformation-dependent antigen, and human antibodies failed to react with the denatured Pgp3 (lane 16).
OmcBc molecules secreted into host cell cytosol but not the OmcB epitopes buried inside the tightly packed outer membrane protein complexes in the EB organisms. The anti-OmcBc labeling was specific, since the signals were removed only by preabsorption with OmcBc and not with other fusion proteins. Second, when the infected cells were permeabilized with SDS, a treatment that can cause leakage of intracellular products by dissolving both lipids and proteins (20, 47), the chlamydial organism-associated OmcBc epitopes became accessible to both anti-OmcBc and -OmcBn antibodies, and at the same time, the soluble OmcBc and CPAF already in the host cell cytosol leaked out of the host cells. Methanol fixation, a method frequently used for treating chlamydia-infected cells prior to immunostaining, may have an SDS-like effect on the membrane vesicles loaded with the OmcBc fragments may promote RB-to-EB conversion. Once OmcBc is released into host cell cytosol, the cytosolic OmcBc has the opportunity to interact with host cell pathways.

The observations that OmcBc is released into host cell cytosol and highly immunogenic during C. trachomatis infection in humans suggest that OmcBc can be targeted for developing rapid diagnosis methods and subunit vaccines. The secreted OmcBc can be conveniently extracted into solutions for antibody recognition, which may allow the development of an immunohistochemistry-based rapid detection of OmcBc in vaginal swab samples from women. The dominant recognition of OmcBc by human antibodies and T cells (23, 25, 46, 60) suggests that OmcBc can access multiple immune processing compartments in humans. Vascularized bacterial antigens are generally more inflammatory and immunogenic (10, 33). If OmcBc is indeed exported into the host cell cytosol via an OMV budding mechanism, the vesiculization may contribute to the enhanced immunogenicity of OmcBc. Human T cell recognition of a highly conserved epitope (450TVYRICVTN RGSACPN462) from OmcBc was associated with chlamydia-induced immunopathology (57), suggesting that anti-OmcBc immune responses can be pathogenic. Thus, caution should be taken when considering OmcBc as a vaccine antigen. Interestingly, the N-terminal region of OmcBc is thought to be exposed on the surface of EBs (41, 56). Thus, it will be worth testing whether immunization with OmcBc or OmcBn can induce protective immunity or exacerbate pathologies in animal models.

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REFERENCES

17. Fadel, S., and A. Eley. 2007. Chlamydia trachomatis OmcBc protein is a