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Interaction of *Brucella abortus* Lipopolysaccharide with Major Histocompatibility Complex Class II Molecules in B Lymphocytes

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Lipopolysaccharide (LPS), a major amphiphilic molecule located at the outer membrane of gram-negative bacteria, is a potent antigen known to induce specific humoral immune responses in infected mammals. LPS has been described as a polyclonal activator of B lymphocytes, triggering the secretion of antibodies directed against distinct sugar epitopes of the LPS chain. But, how LPS is handled by B cells remains to be fully understood. This task appears to be essential for a better knowledge of the anti-LPS humoral immune response. In this study, we examine the internalization of LPS and its interaction with antigen-presenting major histocompatibility complex (MHC) class II molecules in murine and human B-cell lines. By use of immunofluorescence, we observe that structurally different LPSs from *Brucella* and *Shigella* strains accumulate in an intracellular compartment enriched in MHC class II molecules. By use of immunoprecipitation, we illustrate that only *Brucella abortus* LPS associates with MHC class II molecules in a haplotype-independent manner. Taken together, these results raise the possibility that *B. abortus* LPS may play a role in T-cell activation.

Upon infection by gram-negative bacteria, host cells are exposed to antigens that fall into two structurally distinct categories, proteins and lipopolysaccharide (LPS), which exert different activating functions on the immune system. Microbial protein antigens induce both specific cellular and humoral response with memory cells function. Foreign protein antigens are internalized in intracellular compartments of antigen-presenting cells and processed into small peptides (8 to 12 residues), which are then able to associate with major histocompatibility complex (MHC) class II molecules. These antigen-presenting molecules are heterodimers which translocate from the endoplasmic reticulum to the Golgi apparatus before they reach the endocytic pathway, where they bind processed exogenous antigens (17, 35). Then, the complexes are targeted to the cell surface to activate T lymphocytes. Specialized sites for peptide loading have been described both in human and in murine B lymphocytes; these sites appear as multimembrane vesicles and have been termed the compartment for peptide loading (13, 50, 51). In contrast, carbohydrate and glycolipid molecules, such as LPS, are traditionally described as T-independent antigens based on the observations that these molecules are capable of activating B lymphocytes and that they induce the production of antibodies without the apparent contribution of T cells (3, 10, 24). This incapacity to activate T-cell responses is based on the hypothesis that pure polysaccharides and glycolipids fail to bind the MHC class II groove because of their chemical structure (19, 20). However, previous studies showed that bacterial polysaccharides are capable of binding class II molecules in B cells (40, 53). Recently, we demonstrated that *Brucella abortus* smooth LPS and isolated O chain are able to generate sodium dodecyl sulfate (SDS)-resistant MHC class II molecules in murine B lymphocytes (15), a characteristic for MHC class II peptide association (18). In addition, other studies demonstrated the capacity of LPS to activate murine or human T cells in an antigen-presenting-cell-dependent manner (6, 25, 26). Although the activation pathways are not yet characterized, these data strongly suggest a mechanism by which LPS could be presented to T lymphocytes. While many studies have focused on the biological effect of LPS on B cells, none has thus far determined the intracellular fate of LPS in antigen-presenting B cells nor its relationship with antigen-presenting molecules inside cells. Such an approach could give new insights into the central role of LPS in the activation of the immune system which nonspecifically triggers an inflammatory response and induces the secretion of specific anti-O-chain epitope antibodies conferring protection against bacterial infection (8, 9, 37, 38).

In the present study, we investigated the intracellular distribution of three different LPSs (*B. abortus*, *Brucella melitensis*, and *Shigella flexneri*) in human and murine B lymphocytes and their interactions with MHC class I and class II molecules.

The chemical structure of the nontoxic *Brucella* LPS considerably differs from the classical endotoxic enterobacterial LPS, such as *Shigella* LPS, in the polysaccharide chain (O chain), the oligosaccharide core, and the lipid A moiety (31, 41). In contrast, *B. abortus* and *B. melitensis* differ only in their O chain and express an identical lipid A moiety (4). We report that the three different types of internalized LPS accumulate in an MHC class II-positive lysosomal compartment. In addition, we show that *B. abortus* LPS coprecipitates with both murine and human MHC class II molecules in a haplotype-independent

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manner, whereas no association is found with *B. melitensis* and *S. flexneri* LPSs.

MATERIALS AND METHODS

Bacterial strains and LPS extraction. *B. abortus* 2308 (biotype 1 [A serotype]) and *B. melitensis* 16M (biotype 1 [M serotype]) are smooth (S) virulent strains that have been previously described (12). *S. flexneri* serotype 5a has been described elsewhere (47). S-LPS from *B. abortus* 2308 and from *B. melitensis* 16M were prepared simultaneously and in the same conditions as described previously (2). *S. flexneri* serotype 5a LPS was prepared as previously described (52).

Antibodies. Immune serum was obtained from rabbits infected intravenously with 10^9 CFU of *B. melitensis* 16M and bled 15 days later. Absorptions of immune sera with rough *B. abortus* 45/20 bacteria were performed, and their immunoreactivities were tested as previously described (44). Mouse monoclonal antibodies Baps3C/Y (IgM) and Baps1C/Y (IgG3) anti-*Brucella* C/Y O-chain epitope immunoglobulin AC5 (IgAC5) and IgGC20 directed against *S. flexneri* serotype 5a O chain were as previously described (37, 44). The 10.2.16 mouse anti-I-A^k monoclonal antibody (IgG2b) recognizing both $\alpha\beta$ dimers and $\alpha\beta$Ii complexes were previously characterized (5). The rabbit anti-I-A^k antibody recognizing the β chain of MHC class II I-A^k molecule, the mouse H1005/28 anti-$H2^k$ antibody, and the rat anti-mouse Fc receptor antibody (24G2) were provided by N. Barois and L. Leserman (Centre d'Immunologie, Marseille, France). The rat monoclonal M5/114 anti-I-$A^{b,d,q}$, I-$E^{b,k}$ antibody was a gift from R. Germain (National Institutes of Health, Bethesda, Md.). Mouse monomorphic monoclonal anti-HLA-DR antibody, fluorescent-conjugated secondary antibodies, and peroxidase-conjugated secondary antibodies were purchased from Immunotech (Marseille, France), and control isotype antibodies (IgG2b and IgG3) were from Sigma (St. Louis, Mo.). Rabbit anti-cathepsin D antibodies were provided by B. Hoflack (Institut Pasteur, Lille, France).

Cell lines. 2A4 B lymphoma cells expressing MHC class II molecules of the *H-2*^{k} haplotype have been described elsewhere (14). The murine lymphoma LK expressing MHC class II molecules of the *H-2*^{k,d} haplotype was a gift from P. André (Centre d'Immunologie, Marseille, France). Human AT20B, AT24B, and AT80B B cell lines were obtained from Epstein-Barr virus-transformed cell lines. The cell lines were cultured at 37°C under a CO_2 atmosphere in RPMI 1640 (Gibco-BRL, Cergy-Pontoise, France) supplemented with 10% fetal calf serum, 1 mM HEPES, 1 mM sodium pyruvate, 1 mM nonessential amino acids, 2 mM glutamine, and 1 mM penicillin-streptomycin (all from Gibco-BRL).

LPS internalization. Lyophilized LPSs were dissolved in distilled water, sonicated (12 s at 10 to 20 W), and autoclaved. The LPS concentration was adjusted to a 100-μg/ml concentration in cell culture medium before use. Cells (10^6 cells/ml) were incubated with LPS solution for 4 h at 37°C and then extensively washed at 4°C with PBS (pH 7.4)–0.5% bovine serum albumin (BSA; Sigma) before the immunoprecipitation or immunofluorescence experiments.

Immunoprecipitation. Murine and human B cell lines (5×10^6 cells/ml) were incubated with different LPS preparations. After internalization, cells were brought back to 4°C, washed three times with 0.5% PBS-BSA, and lysed for 30 min at 4°C in 1% Nonidet P-40 (NP-40) in PBS (pH 7.4) containing a cocktail of protease inhibitors, allowing the complete detergent solubilization of the membranes (14). After ultracentrifugation, the supernatants were precleared three times by a 2-h incubation with a rabbit anti-mouse IgG (Cappel), preadsorbed on protein A-Sepharose CL4B beads (Pharmacia, Orsay, France). MHC class II LPS was immunoprecipitated by an overnight incubation with 10.2.16, M5/114, anti-HLA-DR; MHC class I LPS was immunoprecipated by overnight incubation with H1005/28; and *Brucella* and *Shigella* LPS was immunoprecipitated by overnight incubation with Baps1C/Y and IgGC20, respectively. Finally, 50 μl of a 50% suspension of protein A-Sepharose beads in PBS was added to supernatants for 1 h at 4°C. Immunoadsorbants were collected by centrifugation, washed three times with 1% NP-40–10 mM Tris-HCl (pH 7.5)–150 mM NaCl–0.5% SDS–0.1% deoxycholate–2 mM EDTA, washed twice more with the same buffer without SDS and deoxycholate, washed twice with 0.5% NP-40–10 mM Tris-HCl (pH 7.5)–150 mM NaCl–2 mM EDTA, and washed twice with 10 mM Tris-HCl (pH 7.5).

Immunoblotting. Sepharose beads were treated with 4% SDS–200 mM dithiothreitol–120 mM Tris (pH 6.8)–0.002% bromophenol blue–20% glycerol, and the supernatants were loaded onto 12% acrylamide SDS-polyacrylamide electrophoresis gels. Samples were then transferred onto Immobilon-P membranes (Millipore, Bedford, Mass.) and blocked in PBS with 5% dry milk and 0.05% Tween 20 (Sigma). Incubation steps with the anti-LPS antibodies were done in the blocking buffer. Anti-LPS antibodies were detected by using peroxidase-conjugated goat anti-IgG antibodies with the enhanced chemiluminescence system (Amersham).

Immunofluorescence. Cells were plated (5×10^5 cells/ml) on glass coverslips precoated with poly-L-lysine (0.1 mg/ml in water; Sigma) for 30 min, fixed at room temperature with 3% paraformaldehyde in PBS (pH 7.4) for 20 min, incubated with 0.1 M glycine in PBS for 10 min, and then permeabilized with 0.1% saponin in PBS. Human B lymphoma cells were saturated with 10% human serum (Sigma) in PBS–0.05% saponin for 20 min before the addition of primary antibodies in 5% human serum–0.05% saponin in PBS for 30 min; they were then extensively washed with 0.05% saponin in PBS and incubated with secondary antibodies for 30 min. Murine B lymphoma cells were saturated with PBS–0.2% BSA for 15 min, followed by an incubation step with 24G2 antibody for 30 min, and then washed and incubated with first primary and then secondary antibodies diluted in 0.2% BSA in PBS. Finally, coverslips were washed, mounted in Mowiol (Hoechst, Frankfurt, Germany), and viewed under a Leica TCS 4DA confocal microscope (Leica Lasertechnik Gmbh, Heidelberg, Germany). A series of two-plane sections of 0.5-μm thickness were monitored. For double-staining experiments, identical optical sections are presented.

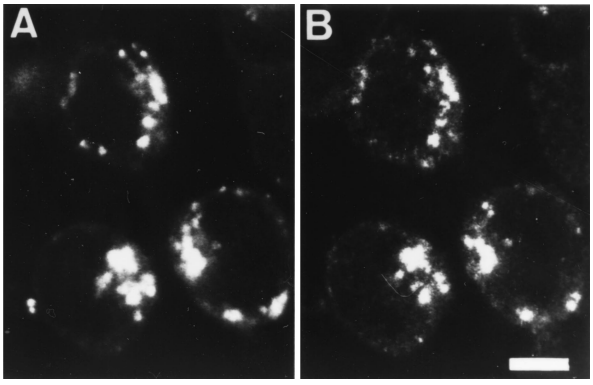

FIG. 1. *B. abortus* LPS accumulates in lysosomal compartments. Human B lymphocytes were incubated for 4 h at 37°C with *B. abortus* LPS. After fixation and permeabilization, double immunofluorescence experiments were analyzed by confocal microscopy. *B. abortus* LPS (A) was found to colocalize with the lysosomal marker cathepsin D (B). Optical sections of 0.5 μm are presented. Bar, 13 μm.

RESULTS

***Shigella* and *Brucella* LPSs accumulate in lysosomal compartments of B lymphocytes.** In order to analyze LPS endocytosis, murine and human B cells were incubated with different LPSs at 37°C for 4 h and processed for immunofluorescence. Figure 1 shows that *B. abortus* LPS colocalizes with cathepsin D, a specific marker for lysosomes. The same immunofluorescence staining pattern was obtained with structurally different LPSs from *B. melitensis* and *S. flexneri* strains (not shown). This suggests that, following interaction with B lymphocytes, LPS molecules are delivered and accumulate into B cell lysosomal compartments independently of the LPS chemical composition.

Heterogeneity and antigenicity of LPS molecules are conserved after LPS internalization. Because of the presence of enzymatic machinery, acidic lysosomal intracellular compartments are specialized sites for the processing of internalized molecules. Since LPS is stored within lysosomes where it may undergo processing, we have compared the structure of LPS before and after internalization. After internalization, cell-associated LPS was precipitated with specific anti-*Brucella* or anti-*Shigella* side-O-chain antibodies and analyzed by immunoblotting in comparison with immunoprecipitated native LPS. Figure 2 demonstrates that LPS can be detected by epitope-specific O-chain antibodies, even after 4 h of lysosomal accumulation in B cells. The ladder-like migration pattern observed corresponds to LPS molecules constituted by different O-polysaccharide lengths, reflecting the molecular weight heterogeneity displayed by LPS molecules in solution (16, 33). However, the presence of LPS degradation products inside B cells could not be completely excluded. We then estimated the amount of LPS within B cells to approximately 20 ng/10^6 cells by comparing the amount of immunoprecipitated internalized LPS with that of immunoprecipitated purified LPS. LPS from *Brucella* or *Shigella* strains gave a comparable signal before (Fig. 2A, lanes b and c, and Fig. 2B, lane a) and after (Fig. 2A, lanes d and e, and Fig. 2B, lane b) internalization, indicating

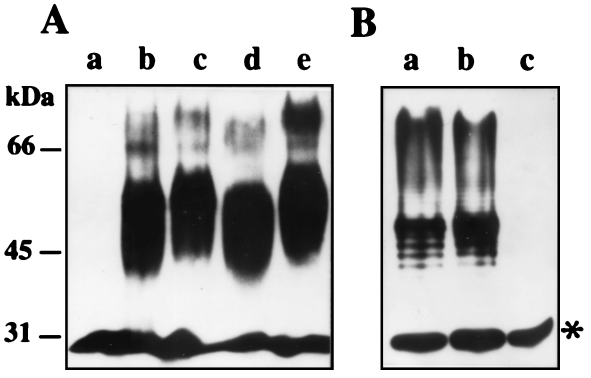

FIG. 2. LPS heterogeneity is conserved after internalization. A total of 0.3 μg of native *B. abortus* (A, lane b), *B. melitensis* (A, lane c), or *S. flexneri* LPS (B, lane a) were immunoprecipitated, and immunoblots were revealed by using the Baps3C/Y anti-*Brucella* O-chain antibody (A) and the IgAC5 anti-*Shigella* O chain (B). In parallel, human B lymphocytes were incubated for 4 h at 37°C with *B. abortus* (A, lane d), *B. melitensis* (A, lane c), or *S. flexneri* LPS (B, lane b). After cell lysis, LPSs were immunoprecipitated, and immunoblots were revealed by use of the respective specific antibodies. In control experiments (A, lane a, and B, lane c), immunoprecipitations were performed in the absence of LPS. The asterisk indicates the light chain of IgG used for immunoprecipitation as revealed by the secondary antibody.

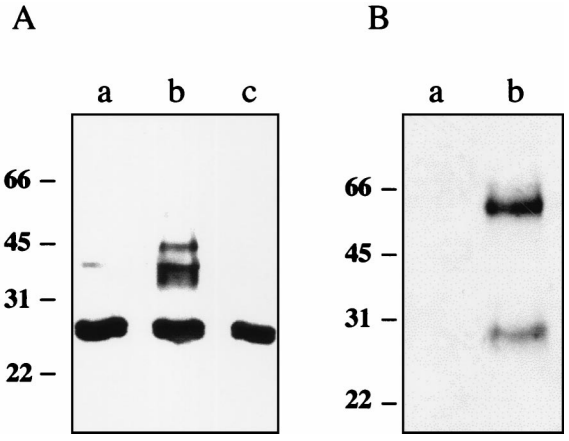

FIG. 4. *B. abortus* LPS associates with murine Ia^k molecules. Murine 2A4 cells were incubated with *B. abortus* LPS (A, lanes b and c, and B, lanes a and b) or with cell culture medium alone (A, lane a) for 4 h at 37°C. Cells were lysed, and immunoprecipitations were processed by using the 10.2.16 anti-class II antibody (lanes a and b) or the control IgG2b isotype antibody (lane c) in panel (A) and the Baps C/Y anti-*Brucella* O-chain antibody (lane b) and the control IgG3 isotype antibody (lane a) in panel B. Immunoblots were revealed by using the mouse anti-O-chain antibody followed by a peroxidase-conjugated goat anti-mouse IgG antibody in panel A and the rabbit anti-MHC class II Iaβ-chain antibody followed by a peroxidase-conjugated goat anti-rabbit IgG antibody in panel B.

that no detectable major modification occurred during the intracellular pathway. We first concluded that internalized LPS kept its complete general structure (lipid A linked to the O chain via the core) within B cells, since we know that the lipid A moiety is essential for both entry and migration of LPS in SDS gels. Secondly, the LPS immunodominant moiety (the O chain) was not degraded, since we observed the conservation of its length heterogeneity and antigenicity inside B-cell lysosomal compartments.

***Brucella* and *Shigella* LPSs accumulate in MHC class II-positive compartments in human B cells.** B cell lines are known to contain MHC class II-positive compartments (MIICs), specialized in the processing, the loading of antigenic peptides onto class II molecules, and the presentation of exogenous peptides to T lymphocytes (28). The MIICs are related to lysosomal compartments in human Epstein-Barr virus-transformed B-cell lines (35). To determine whether LPS accumulates in the MIICs, we analyzed by immunofluorescence the distribution of LPS and HLA-DR molecules in human B lymphocytes. Figure 3 shows that, after 4 h of incubation with B cells, *B. abortus* LPS concentrates within vesicles enriched in class II molecules. A similar staining profile was observed with *B. melitensis* and *S. flexneri* LPSs (data not shown), confirming that the fate of LPS in B lymphocytes is independent of structural differences between these glycolipids. Taken together, Fig. 1 and 3 show that LPS accumulates within compartments containing both cathepsin D and HLA-DR, suggesting that these intracellular compartments correspond to MIICs.

In both human and murine B lymphocytes, *B. abortus* LPS binds to MHC class II molecules. Because LPS accumulates in MHC class II-enriched vesicles, where molecular association of processed proteic antigens with HLA-DR occurs, we searched for possible interactions between LPS and class II molecules. In the murine B cell from the $H\text{-}2^k$ haplotype, *B. abortus* LPS was revealed after immunoprecipitation of MHC-class II molecules (Fig. 4A). In addition, after LPS immunoprecipitation, two bands associated with MHC class II molecules were detected by specific anti-IA^k antibodies (Fig. 4B): the former (ca.

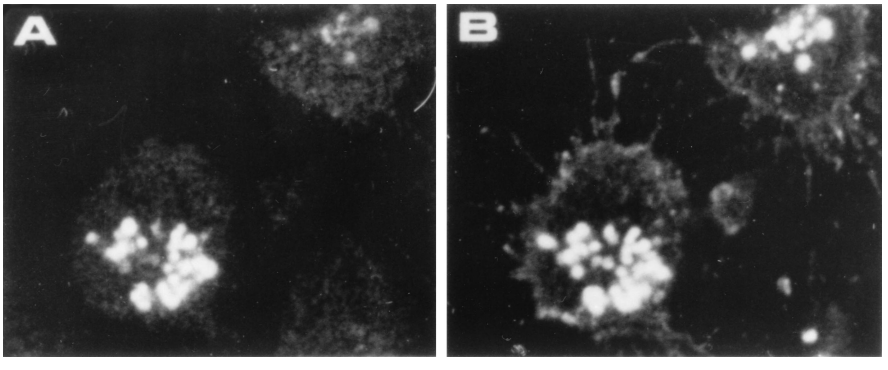

FIG. 3. LPS concentrates in MHC class II-enriched intracellular compartments. Human AT24B B lymphocytes were incubated for 4 h at 37°C with *B. abortus* LPS. After fixation and permeabilization, double immunofluorescence experiments were analyzed by confocal microscopy. (A) LPS was stained by a rabbit serum anti-LPS followed by a Texas red-conjugated anti-rabbit antibody. (B) HLA-DR molecules recognized by an anti-HLA-DR mouse monoclonal antibody were revealed by use of a donkey anti-mouse IgG coupled to fluorescein. Bar, 10 μm.

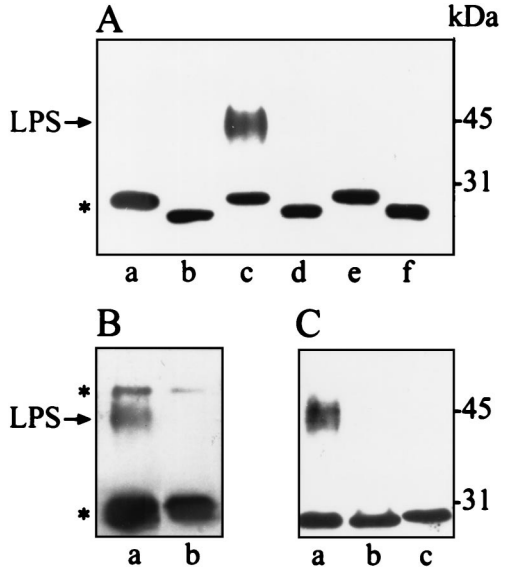

FIG. 5. *B. abortus* LPS coprecipitates with MHC class II molecules. (A) 2A4 B cells were incubated with *B. melitensis* (lanes a and b), *B. abortus* (lanes c and d), or *S. flexneri* (lanes e and f) LPS. Class II molecules were precipitated with the 10.2.16 anti-IA^k (lanes a, c, and e) antibody, and class I molecules were precipitated with the anti-$H2^k$ antibody (lanes b, d, and f). (B) Murine LK B cells were incubated for 4 h at 37°C with *B. abortus* (lane a) or *B. melitensis* (lane b) LPS, and class II molecules were immunoprecipitated with the M5114 antibody. (C) Human AT24B B lymphocytes were incubated for 4 h at 37°C with *B. abortus* (lane a), *B. melitensis* (lane b), or *S. flexneri* (lane c) LPSs. After cell lysis, HLA-DR molecules were precipitated. Detection of class II molecule-associated LPS was performed as described for Fig. 2. The asterisk indicates the light chain of IgG used for immunoprecipitation of the class II molecules in panels B and C as well as the heavy chain of IgG in panel B as revealed by the secondary antibody.

30 kDa) corresponds to the free Iaβ chain, and the latter (ca. 60 kDa) shows that LPS has induced the generation of SDS-resistant MHC class II dimers characteristic of the formation of MHC class II molecule-antigen complexes (13, 15, 17). We extended this analysis to human B-lymphoma and murine B-cell lines from the $H\text{-}2^d$ haplotype (Fig. 5). Strikingly, *B. abortus* LPS coprecipitated with class II molecules independently of both the MHC haplotype and the cell species origin. In addition, this interaction appeared to be restricted to MHC class II molecules, since *B. abortus* LPS was not detected after immunoprecipitation of MHC class I molecules (Fig. 5A, lanes b, d, and f). In contrast, no association was found neither with *B. melitensis* LPS (Fig. 5A, lane a; Fig. 5B, lane b; Fig. 5C, lane b) or with *S. flexneri* LPS (Fig. 5A, lane e, and Fig. 5C, lanes b and c), rendering the complex formation process specific to LPS from *B. abortus*. Given the fact that *B. melitensis* LPS differs from *B. abortus* LPS by its O-chain part and *S. flexneri* LPS varies in the lipid A, core, and O-chain moiety, these results indicate that the interaction depends on the chemical structure of LPS and leads to the hypothesis that the *B. abortus* LPS O-chain moiety plays a central role in the association with MHC class II antigen-presenting molecules.

DISCUSSION

LPS represents the major antigen as well as the main toxic component of gram-negative bacteria. This endotoxin, associated with bacteria or release by microorganisms during their death or growth as "free LPS" or as "outer-membrane-complexed LPS" (46), exerts powerful pathophysiological effects in infected organisms by activating specific host cells. Among the most important target cells of LPS are the B lymphocytes, which are stimulated to proliferate, differentiate, and secrete antibodies after interaction with endotoxins. The events leading to B-cell activation, LPS cell recognition, transduction signaling cascade, and the subsequent biological effects of this endotoxin have been largely investigated, and studies have pointed out the lipid A moiety of enterobacteria as the biological active part of endotoxin (49). Even though the structure of lipid A is highly conserved among enterobacteria such as *Shigella* spp., *Salmonella* spp., or *Escherichia coli*, its structure is quite distinct in organisms such as *Brucella* spp. Consequently, *Brucella* LPS displays a very low endotoxic activity (29, 42) but conserves its immunogenic properties, inducing the secretion of antibodies directed against a variety of its carbohydrate epitopes (30).

Until now, the paradigms of antigen processing and presentation have been limited to data mainly generated from proteins (18). Conversely, critical investigations concerning the uptake, transport, processing, and presentation of carbohydrates and lipids have so far been addressed in a marginal manner. In this respect, intracellular trafficking of LPS molecules has been investigated in macrophages and showed that LPS distributed in the cytoplasm, nucleus, and different endocytic compartment of cells (15a, 21, 43). The divergence of these results could be explained by the different experimental procedures, including the use of different target cells, various LPS preparations, and diverse methods of internalization. Indeed, Kitchens et al. recently demonstrated that the route of LPS trafficking depends upon its internalization pathways (CD14 dependent or independent) and its aggregation state (22, 23). Here, we found that LPSs are delivered, independently from their chemical composition, to lysosomal compartments of B cells, as demonstrated by the strong colocalization observed with cathepsin D. In this investigation, we estimated that incubation with 100 μg of LPS per ml for 4 h per 10^6 B lymphocytes, which are nonprofessional phagocytes and poor endocytic cells, gave an intracellular LPS signal comparable to that observed in phagocytic and epithelial cells after the processing of a few intracellular bacteria (one to five) within lysosomes (39, 48). Compared to the LPS concentration in the culture medium, the amount of LPS ingested by B lymphocytes is relatively small and has been estimated to 20 ng/10^6 B cells (Fig. 3), indicating that LPS endocytosis in B cells is not efficient, and thus the question whether or not LPS is internalized by receptor-mediated endocytosis in B lymphocytes remains to be elucidated. However, this amount is commensurate with the quantities of LPS detected in association with cells after infection (27, 34). In addition, the concentration of bacteria in infected tissue (e.g., lymph node, spleen, fetus, etc.) may reach 10^{13} per g (1), suggesting the presence of a high concentration of LPS in the infected host. In conclusion, the concentration of LPS used and detected in our system may correctly mimic the natural conditions of infection.

Numerous studies about LPS processing in macrophages or in polymorphonuclear cells have focused on the biologically active part of LPS and supported the idea that LPS is detoxified after deacylation and dephosphorylation of the lipid A by specific enzymatic mechanisms (32, 36). We recently showed that, in macrophages, LPS is routed to lysosomes after binding to the cell surface, where it accumulates (up to 24 h) without undergoing modifications either in the structure or in antigenicity and heterogenicity. In the present study, we confirm that in B cells specialized in anti-O-chain antibody production, the LPS detected in the acid lysosomal compartment after 4 h of incubation has kept its structural integrity and its O-chain antigenicity. However, this method cannot give more details

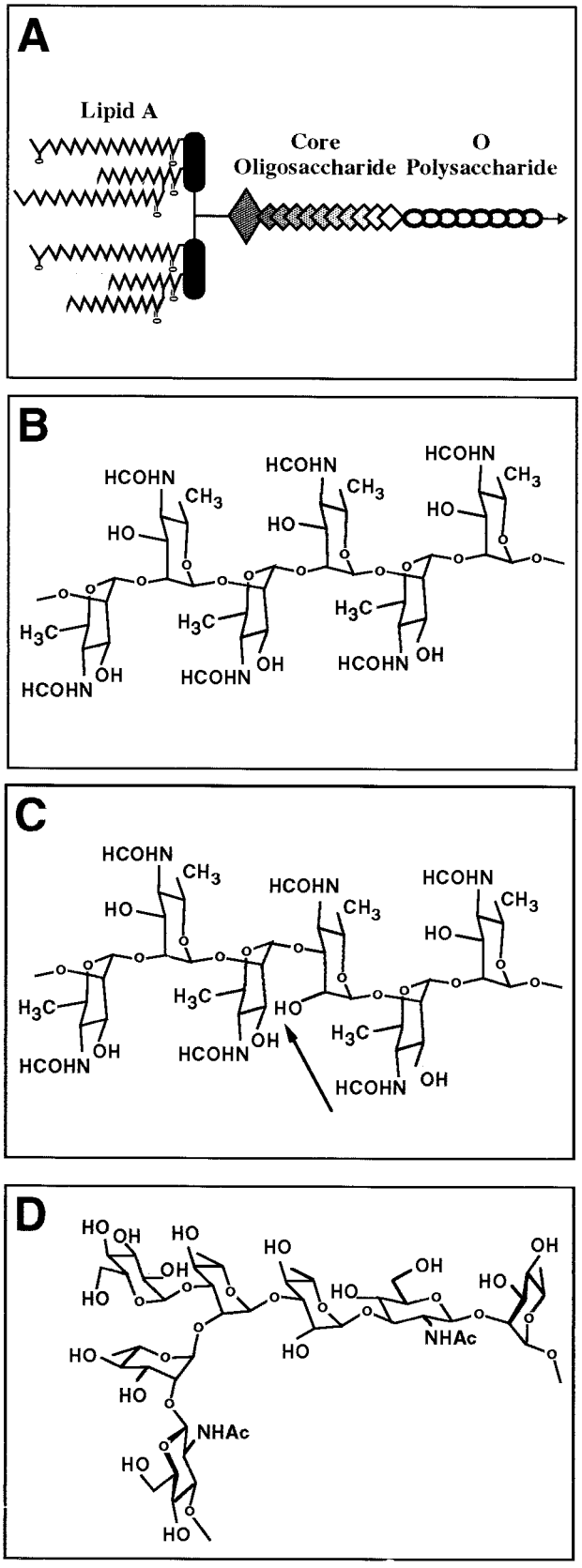

FIG. 6. Chemical models of LPS (A), *B. abortus* 2308 (B), *B. melitensis* 16M (C), and *S. flexneri* 5a (D) O chains. The *B. abortus* O chain adopts a rod-like structure with a cross-section of ca. 10 Å, with a distance of 20 Å separating the first and sixth formamido groups. The presence of an $\alpha_{1,3}$ linkage at every fifth residue in the *B. melitensis* O chain introduces a serious kink into the rod-like structure (showed by an arrow in panel C).

about the fine structure of LPS and about the eventual generation of LPS degradation products.

In addition to the fact that LPS accumulates in a lysosomal compartment, we found that these compartments were highly enriched in MHC class II molecules resembling MIICs. The MIICs are specialized sites for peptide loading, and the presence of LPS in these compartments might have clear implications in the function of LPS inside B lymphocytes. We have previously shown that *B. abortus* LPS and its isolated O chain, but not lipid A, were able to generate SDS-resistant MHC class II molecules in murine B lymphocytes (15), a characteristic of MHC class II associated with processed antigens (17). In this work, we demonstrate that only *B. abortus* LPS associates with MHC class II molecules in a haplotype- and species-independent manner and that *B. abortus* LPS-class II molecule binding is very likely to occur in an intracellular compartment enriched in class II molecules. The observation that *B. melitensis* LPS can reach the MHC class II compartment but does not bind to class II molecules suggests that the binding of LPS by the MHC class II is dependent of a particular O-chain structure (7). Indeed, the O-polysaccharide chain of *B. abortus* consists of an unbranched linear homopolymer of a $\alpha_{1,2}$-linked 4-formamido-4,6-dideoxy-D-mannose (*N*-formylperosamine) residues, while the O chain of *B. melitensis* consists of a repeating block of five *N*-formylperosaminyl residues, four that are $\alpha_{1,2}$-linked and one that is $\alpha_{1,3}$ linked (Fig. 5). In its best-ordered minimum-energy configuration, the *B. abortus* O-chain antigen adopts a rod-like helix structure with a cross section of ca. 10 Å with the $\alpha_{1,2}$-linked oxygen-carbon atoms of the perosamine sugar building the core of the rod with a distance of about 20 Å separating the first and the sixth formamido groups and the 6-deoxy and 4-formamido groups (45). This structural and chemical conformation of the *B. abortus* antigen seems to fulfill the binding requirements of MHC class II, whose open-ended groove and generic contacts along the antigen binding site permit the coupling of longer molecules, such as medium-size peptides and denatured proteins (11). However, the $\alpha_{1,3}$ linkage of every fifth *N*-formylperosamine residue within the *B. melitensis* O chain introduces a serious kink, which distorts the rod-like helix structure (Fig. 6). This small but significant chemical difference seems to be enough to prevent the binding of *B. melitensis* LPS to class II molecules and suggests that at least five to six $\alpha_{1,2}$-linked *N*-formylperosamine residues are required for binding. The fact that a restricted population of *B. abortus* LPS molecules coprecipitated with class II molecules (Fig. 4 and 5) suggests that this microbial antigen might be subjected to size selection by B lymphocytes. We have recently shown that in macrophages, *B. abortus* LPS follows the endocytic pathway and finally reaches the cell plasma membrane by an unidentified recycling mechanism. Along this pathway the structure, the antigenicity, and the heterogeneity of LPS are preserved (15a). In addition, this intact recycled LPS was found clustered with MHC class II molecules at the surface of the macrophages (unpublished data). These data, together with the present findings, lead to the assumption that the *B. abortus* LPS-MHC class II molecule complexes may be routed to the cell surface for an eventual presentation of LPS to the T cells. Alternatively, instead of activating T cells, LPS, by interacting with MHC-class II molecules, could interfere with the ability of B cells to present exogenous proteic antigen in an

MHC class II-restricted context. Further studies are needed to clarify the exact role of *B. abortus* LPS in MHC class II trafficking and function.

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