O Antigen Protects *Bordetella parapertussis* from Complement[▽]

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Bordetella pertussis, a causative agent of whooping cough, expresses BrkA, which confers serum resistance, but the closely related human pathogen that also causes whooping cough, Bordetella parapertussis, does not. Interestingly, B. parapertussis, but not B. pertussis, produces an O antigen, a factor shown in other models to confer serum resistance. Using a murine model of infection, we determined that O antigen contributes to the ability of B. parapertussis to colonize the respiratory tract during the first week of infection, but not thereafter. Interestingly, an O antigen-deficient strain of B. parapertussis was not defective in colonizing mice lacking the complement cascade. O antigen prevented both complement component C3 deposition on the surface and complement-mediated killing of B. parapertussis. In addition, O antigen was required for B. parapertussis to systemically spread in complement-sufficient mice, but not complement-deficient mice. These data indicate that O antigen enables B. parapertussis to efficiently colonize the lower respiratory tract by protecting against complement-mediated control and clearance.

The major component of the outer leaflet of gram-negative bacteria, lipopolysaccharide (LPS), is composed of three major regions; a lipid A, a core oligosaccharide, and an O polysaccharide (O antigen) (12). Most biological effects of LPS have been attributed to the immunostimulatory properties of lipid A (35, 44); however, O antigen plays important roles in protection against host immune mechanisms, such as complement-mediated killing, and antimicrobial peptide-mediated bactericidal effects (9, 24, 38, 39, 45, 48, 52, 53). For example, the shortened O antigen of serum-sensitive strains of *Pseudomonas aeruginosa* is associated with increased C3 deposition (47). The presence of O antigen on *Klebsiella pneumoniae* LPS appears to have no effect on C3 deposition or its ability to cause pneumonia, although its presence does increase serum resistance in vitro (1, 16). In addition, *P. aeruginosa* and *Yersinia entercolitica* O antigens affect the expression and/or proper function of other virulence factors (4, 6, 10, 38). These examples illustrate that there is considerable variation in the function of the O antigen portion of the LPS among different bacterial pathogens (12, 35).

Bordetella parapertussis and *Bordetella pertussis* are the causative agents of whooping cough (34). Although these pathogens are very closely related (17, 36, 56), there is substantial variation in LPS structures between them (2, 3, 17, 42, 43). *B. pertussis* produces a lipo-oligosaccharide containing lipid A and a branched-chain core oligosaccharide with a complex trisaccharide modification, but completely lacks O antigen due to a 20-kb deletion in the *wbm* locus responsible for O-antigen synthesis (36). *B. parapertussis* produces an LPS molecule that has a distinct lipid A and a core oligosaccharide lacking the

trisaccharide modification, but includes an O antigen (42, 43, 55). Interestingly, both of these pathogens are commonly found in the human population, indicating that O antigen is not necessary for human infection (34). However, a defined role for O antigen during *B. parapertussis* infection has not yet been described clearly.

Previous studies have shown that compared to the wild-type strain, an isogenic mutant of *B. parapertussis* lacking several genes necessary for O antigen synthesis (Δ*wbm*) is severely defective in colonization of the respiratory tracts of BALB/c mice when given in a low-dose inoculum (1,000 CFU) and is more sensitive to in vitro serum-mediated killing (9). This increased sensitivity to in vitro serum exposure was abrogated by prior complement depletion, indicating that O antigen is protective against complement-mediated killing in vitro (9).

To investigate the role of O antigen during infection, we used a standard high-dose inoculation regimen (18, 22, 23, 27, 32). This regimen allowed *B. parapertussis* Δ*wbm* to persist in the respiratory tract for 28 days, similar to the situation for wild-type bacteria. Interestingly, the O antigen mutant showed a defect only during the first week of infection, suggesting that the defect is due to an increased susceptibility to, or an increased activation of, an innate immune function. While *B. parapertussis* Δ*wbm* levels were lower than wild-type *B. parapertussis* levels in the absence of neutrophils or macrophages, this defect was not observed in mice deficient in complement component C3. In vitro assays showed that O antigen inhibited complement component C3 deposition on *B. parapertussis*. In RAG$^{-/-}$ mice, O antigen was required for the systemic spread of *B. parapertussis* but was not required following complement depletion. This result suggests that O antigen facilitates the systemic spread of *B. parapertussis* via evasion of complement. Together, our data indicate that the O antigen of *B. parapertussis* protects against complement deposition and complement-mediated killing, allowing for efficient colonization of the murine host.

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MATERIALS AND METHODS

Bacterial strains and growth. *B. parapertussis* strain CN2591 and an isogenic mutant lacking O antigen, CN2591Δ*wbm*, have previously been described (42) and were maintained on Bordet-Gengou agar (Difco, Sparks, MD) containing 10% sheep's blood (Hema Resources, Aurora, OR) with 20 μg/ml streptomycin. For inoculation, the bacteria were grown overnight at 37°C in Stainer-Scholte broth (26, 54) to mid-log phase and diluted in phosphate-buffered saline (PBS; OmniPur, Gibbstown, NJ) to a concentration of 1.0×10^7 CFU/ml (23, 28, 60, 61).

Animal experiments. Four- to 6-week-old C57BL/6, CD11b$^{-/-}$, μMT, RAG1$^{-/-}$, and C5$^{-/-}$ mice were obtained from Jackson Laboratories (Bar Harbor, ME) and were bred in a *Bordetella*-free environment. C3 knockout (C3$^{-/-}$) mice were a kind gift from Rick Wetsel and have previously been described (15). All mice were maintained in housing facilities approved by Pennsylvania State University and were closely monitored in accordance with institutional policies and IACUC regulations. For inoculation, mice were lightly sedated with 5% isofluorane in oxygen and 50 μl of PBS containing 5×10^5 CFU of the indicated bacteria was pipetted onto the tips of the external nares (28, 62). Groups of three or four animals were sacrificed via CO$_2$ inhalation on the indicated days for postmortem dissection of the lungs, trachea, nasal cavity, spleen, liver, and/or kidneys, as indicated. We quantified the bacterial colonization by homogenizing each tissue in PBS, serial plating, and subsequently counting colonies (22). Serum was collected via postmortem cardiac puncture from μMT mice. Survival curves were generated by infection of groups of 15 to 20 RAG1$^{-/-}$ mice with either CN2591 or CN2591Δ*wbm* with or without cobra venom factor (CVF) treatment, as indicated (50). Mice suffering from lethal bordetellosis, as determined by severe hunched posture, ruffled fur, extremely labored breathing, and apathy, were euthanized to prevent unnecessary suffering (31, 62).

Depletion of immune factors and quantification of leukocytes. For neutrophil depletion, mice were intraperitoneally injected with 1 mg αLy-6G antibody from the hybridoma RB6-8C5 48 h prior to inoculation (27, 51). The depletion of 99% of neutrophils was confirmed via blood smear. For complement depletion, mice were intraperitoneally injected with 5 U of CVF in PBS (Sigma, St. Louis, MO) at 26 and 24 h prior to inoculation, and every 5 days thereafter until the completion of the experiment (50). For alveolar macrophage depletion, mice were given an intranasal dose of 100 μl of clodronate liposomes (Roche Diagnostics, Mannheim, Germany) 48 h prior to inoculation (11). The presence or absence of alveolar macrophage or neutrophils in the lungs was determined via visual identification of cells from bronchoalveolar lavage fluid spun onto slides via Cytospin and then stained with modified Wright-Giemsa stain (Fisher Scientific, Kalamazoo, MI).

C3 deposition assay. Approximately 4×10^8 CFU of mid-log-phase CN2591 and CN2591Δ*wbm* bacteria were harvested by centrifugation and incubated at 37°C for 30 min with 20% naïve, antibody-deficient, complement-active or heat-inactivated serum collected from μMT mice. Samples were washed twice with PBS, incubated for 30 min on ice with fluorescein isothiocyanate (FITC)-labeled goat anti-mouse C3 (MP Biomedicals), washed twice with cold PBS to remove unbound antibody and then analyzed by flow cytometry. Samples were run in triplicate, and the experiment was repeated independently.

Serum killing assay. Naïve C57BL/6 and C3$^{-/-}$ mice were bled, and the pooled blood was placed on ice for 30 min. Samples were spun, and the serum was extracted and diluted to the indicated concentrations. One thousand CFU of mid-log-phase CN2591 and CN2591Δ*wbm* in 5 μl of PBS were incubated with 45 μl of diluted serum for 1 h at 37°C, followed by subsequent plating and colony count. Samples were run in triplicate, and the experiment was repeated independently.

In vivo bioluminescence imaging of bacterial colonization. The *B. parapertussis* strains C2591 and C2591Δ*wbm* were rendered bioluminescent by the chromosomal insertion of pSS4266 to produce strains BPS1766 (*B. parapertussis*::luciferase) and BPS1768 (*B. parapertussis* Δ*wbm*::luciferase), respectively. Briefly, pSS4266 is a derivative of the chromosomally integrating promoter assay vector pSS3110 (57) in which the *lacZYA* operon has been replaced by the *luxCDABE* operon of *Photorhabdus luminescens*, derived from pUTminiTn5km*lux* (21). Luciferase expression in pSS4266 is driven by the *fha* promoter of *Bordetella pertussis*. BPS1766 and BPS1768 were used to inoculate littermate RAG1$^{-/-}$ mice intranasally as described above. Mice were subsequently imaged daily in an IVIS-100 apparatus (Xenogen Corp.) according to the manufacturer's instructions.

Statistical analysis. Student's two-tailed *t* test was used to determine statistical significance between experimental groups. Values of $P \leq 0.05$ were considered significant.
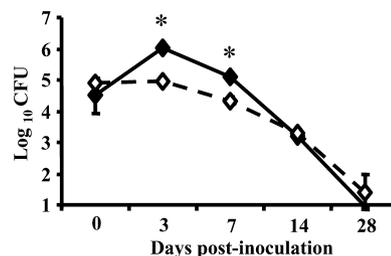

FIG. 1. Colonization of mouse lungs by *B. parapertussis* and *B. parapertussis* Δ*wbm* over time. Groups of three 4- to 6-week-old C57BL/6 mice were inoculated with 5×10^5 CFU of *B. parapertussis* (filled diamonds) or *B. parapertussis* Δ*wbm* (open diamonds). The number of CFU recovered from the lungs at each indicated time point is expressed as the log$_{10}$ means ± standard errors (error bars). *, $P \leq 0.05$. The limit of detection is log$_{10}$1, indicated as the *y* intercept.

RESULTS

O antigen contributes to *B. parapertussis* colonization of the murine respiratory tract. Previous studies have shown that *B. parapertussis* lacking O antigen (*B. parapertussis* Δ*wbm*) is nearly cleared from the lower respiratory tract within 3 days using a low-dose (1,000 CFU) inoculation regimen in BALB/c mice, while *B. parapertussis* is able to increase in number (9). To more thoroughly examine interactions between O antigen and the immune response, we used a high-dose inoculation regimen that distributes the bacteria throughout the respiratory tract in numbers that rigorously test the innate and adaptive immune responses (9, 22, 28, 62). C57BL/6 mice were inoculated with 5×10^5 CFU of *B. parapertussis* or *B. parapertussis* Δ*wbm* and sacrificed on day 0, 3, 7, 14, or 28 postinoculation. Mice dissected 10 min postinoculation had approximately 10^5 CFU of *B. parapertussis* or *B. parapertussis* Δ*wbm* in their lungs (Fig. 1). *B. parapertussis* levels increased over the first few days to 10^6 CFU in the lungs and decreased steadily over the subsequent 3 weeks to approximately 10^5 CFU, 10^3 CFU, and 10^1 CFU by days 7, 14, and 28 postinoculation, respectively (Fig. 1). *B. parapertussis* Δ*wbm* levels in the lungs were approximately 10-fold lower than that of *B. parapertussis* on days 3 and 7 postinoculation, but both *B. parapertussis* and *B. parapertussis* Δ*wbm* were found at similar levels on days 14 and 28 postinoculation (Fig. 1). The 90% lower levels of the O antigen-deficient strain on only days 3 and 7 postinoculation, but not later days, suggest that the O antigen of *B. parapertussis* contributes to infection at relatively early stages but has little effect on eventual clearance.

O antigen does not contribute to colonization in the absence of complement. In order to investigate the immune mechanism that O antigen protects against, we blocked various innate immune functions and examined bacterial numbers 3 days postinoculation. Neutrophils or alveolar macrophages were depleted from wild-type mice with αLy6G antibody or clodronate liposome treatment, respectively (11, 27, 51). These mice were then inoculated with *B. parapertussis* or *B. parapertussis* Δ*wbm* as described above and dissected 3 days postinoculation. In neutrophil-depleted mice, *B. parapertussis* reached approximately $10^{6.5}$ CFU, while *B. parapertussis* Δ*wbm* reached levels of $10^{5.5}$ CFU in the lungs, a 10-fold defect relative to wild-type bacteria (Fig. 2). In addition, there was no difference in neu-
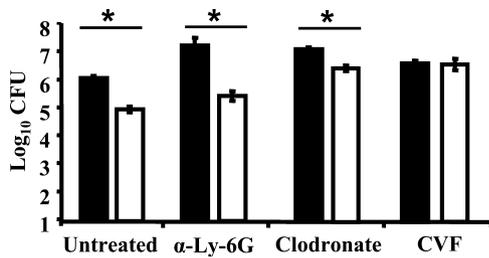
FIG. 2. Colonization of mouse lungs by *B. parapertussis* and *B. parapertussis* Δ*wbm* upon depletion of neutrophils, alveolar macrophages, or complement. Groups of three 4- to 6-week-old C57BL/6 mice were left untreated or were treated with α-Ly-6G antibodies, clodronate liposomes, or CVF prior to inoculation with 5×10^5 CFU of *B. parapertussis* (filled bars) or *B. parapertussis* Δ*wbm* (open bars), as indicated. The number of CFU recovered from mouse lungs at day 3 postinoculation is expressed as the $\log_{10}$ means ± standard errors (error bars). The asterisk indicates $P \leq 0.05$. The limit of detection is $\log_{10}1$, indicated as the *y* intercept.

trophil accumulation in the lungs of wild-type mice at 12 h and 24 h postinoculation with *B. parapertussis* or *B. parapertussis* Δ*wbm* (data not shown). This result indicated that O antigen contributes to colonization even in the absence of neutrophils and therefore must protect *B. parapertussis* from some other immune function (Fig. 2). Similarly, the O antigen-deficient strain was also defective in its ability to colonize alveolar macrophage-depleted mice (Fig. 2). Unfortunately, mice depleted of both neutrophils and alveolar macrophages succumbed to sham inoculations consisting of sterile PBS and, therefore, the effect of O antigen on the ability of *B. parapertussis* to colonize doubly depleted hosts was not assessed. These results suggest that the early defect of the O antigen-deficient strain is not simply the result of increased susceptibility to neutrophil- or macrophage-mediated clearance alone, but is instead due to sensitivity to some other innate immune function.

Since *B. parapertussis* Δ*wbm* is susceptible to complement in vitro (9), we sought to determine whether complement was responsible for the defect of *B. parapertussis* Δ*wbm*. CVF was given to deplete complement in wild-type mice 26 and 24 h prior to inoculation (50). Both *B. parapertussis* and *B. parapertussis* Δ*wbm* were recovered at approximately $10^{6.5}$ CFU on day 3 postinoculation in the lungs of CVF-treated mice, a level similar to that for untreated mice inoculated with wild-type *B. parapertussis* (Fig. 2). These results suggest that the lack of O antigen leaves the bacteria more susceptible to complement-mediated control in vivo. Furthermore, there appears to be no significant role for O antigen in the absence of complement during the first week of infection (Fig. 2).

O antigen prevents C3 deposition on the surface of *B. parapertussis*. Since O antigen appeared to protect *B. parapertussis* against complement-mediated control, we sought to examine the ability of C3 to bind to the surface of *B. parapertussis* and *B. parapertussis* Δ*wbm*. Bacteria were incubated with 20% naïve, antibody-deficient serum that was either maintained as complement active or heat-inactivated. Surface bound C3 was detected by FITC-labeled anti-C3 antibodies. *B. parapertussis* incubated with serum showed a similar amount of FITC-positive staining as *B. parapertussis* incubated without serum or

with heat-inactivated serum (Fig. 3), indicating that wild-type *B. parapertussis* was able to avoid complement deposition. Interestingly, *B. parapertussis* Δ*wbm* showed a 50-fold increase in C3-positive cells (Fig. 3F) when incubated with complement active serum compared to the number of C3-positive *B. parapertussis* cells with similar treatment (Fig. 3E). C3 deposition on the O antigen mutant was not observed upon incubation with heat-inactivated serum (Fig. 3G and H). Together, these data indicate that the presence of O antigen prevents C3 deposition on *B. parapertussis*.

To determine whether the deposition of C3 caused subsequent complement-mediated killing of *B. parapertussis* Δ*wbm*, 1,000 CFU of either *B. parapertussis* or *B. parapertussis* Δ*wbm* was incubated with various concentrations of complement active serum from wild-type mice or complement-deficient serum from C3$^{-/-}$ mice. *B. parapertussis* Δ*wbm* was significantly more sensitive to wild-type serum at concentrations of 10% serum or higher than complement-deficient serum (Fig. 3I). These results indicate that O antigen protects *B. parapertussis* from C3 deposition and subsequent complement-mediated killing.

C3 causes the in vivo defect of the O antigen-deficient strain of *B. parapertussis*. In order to dissect the specific components of complement that limited *B. parapertussis* Δ*wbm* levels by day 3 postinoculation, we inoculated mice lacking different aspects of the complement cascade. C3$^{-/-}$ mice are unable to produce complement component C3, the protein required for positive feedback and amplification of both classical and alternative complement activation pathways as well as opsonization and subsequent formation of the membrane attack complex (MAC) on bacterial cells (15). C3$^{-/-}$ mice harbored similar bacterial loads of *B. parapertussis* and *B. parapertussis* Δ*wbm*, indicating that C3 is required to reduce the levels of *B. parapertussis* Δ*wbm* (Fig. 4). C5$^{-/-}$ mice lack complement component C5, which is required to form the MAC but not for the positive feedback, amplification, and opsonization via C3 deposition. CD11b$^{-/-}$ mice are able to form the MAC, but lack the complement receptor type III (CR3), which is known to be important in host response to LPS and is found on macrophages, neutrophils, dendritic cells, and natural killer cells (37, 40, 46, 58). *B. parapertussis* Δ*wbm* levels were 10-fold lower than those of wild-type bacteria in both C5$^{-/-}$ and CD11b$^{-/-}$ mice, indicating that individually, these complement factors are not required for the more efficient control of *B. parapertussis* Δ*wbm* (Fig. 4). These results further indicate that the defect in colonization of the O antigen mutant is a result of its increased susceptibility to complement activities that require C3, but not C5 or CR3 individually.

O antigen is required for the systemic spread of *B. parapertussis*. Since O antigen is known to facilitate systemic colonization in other infection models, we sought to determine the role of O antigen in the systemic spread of *B. parapertussis* (30, 49). RAG1$^{-/-}$ mice were infected with either *B. parapertussis*::luciferase or *B. parapertussis* Δ*wbm*::luciferase, and the colonization was visualized on day 21 post-inoculation (Fig. 5A and B). Mice infected with *B. parapertussis*::luciferase showed high levels of colonization in the thoracic cavity and colonization in the upper abdominal cavity. In contrast, mice infected with *B. parapertussis* Δ*wbm*::luciferase showed colonization only in the respiratory tract. To determine the numbers of *B. parapertussis* or *B.*
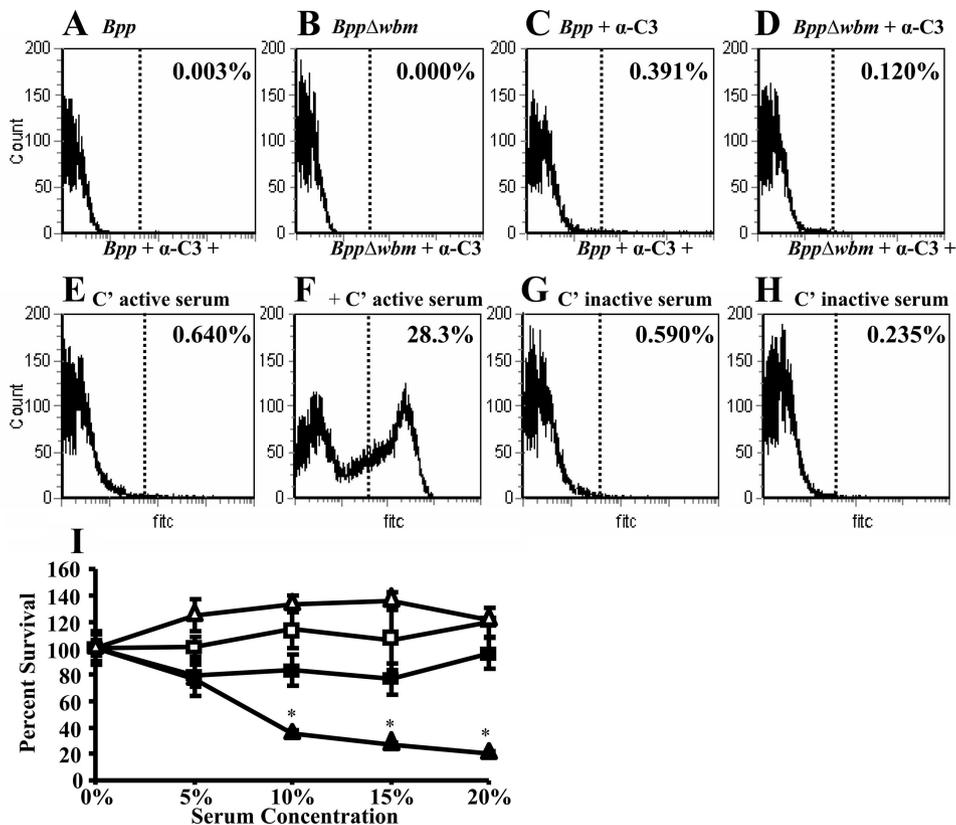
FIG. 3. Flow cytometry analysis of C3 deposition on *B. parapertussis* and *B. parapertussis* Δ*wbm*. Approximately 4×10^8 CFU of *B. parapertussis* (A, C, E, and G) or *B. parapertussis* Δ*wbm* (B, D, F, and H) was harvested from mid-log-phase cultures, incubated at 37°C without serum (A to D), with 20% complement active naïve mouse serum (E and F), or with 20% heat-inactivated naive mouse serum (G and H) for 30 min. Samples either were left unstained (A and B) or were labeled with FITC-anti-mouse C3 antibodies (C to H) and analyzed by flow cytometry. The percentage of FITC-positive cells is indicated as the average of three replicates. (I) *B. parapertussis* (squares) or *B. parapertussis* Δ*wbm* (triangles) were exposed to complement active (closed) or complement-deficient (open) mouse serum at the indicated concentrations for 1 h. The average percent survival of three replicates is shown. Error bars represent standard errors. ∗, $P \leq 0.05$.

parapertussis Δ*wbm* isolates in systemic organs, groups of four to six RAG1$^{-/-}$ mice were infected with either *B. parapertussis* or *B. parapertussis* Δ*wbm* and then dissected on day 17 postinoculation. Significantly higher numbers of *B. parapertussis* isolates were found in the respiratory tract compared to the numbers of *B. parapertussis* Δ*wbm* isolates. Additionally, *B. parapertussis* had spread systemically in all RAG1$^{-/-}$ mice and was found in the spleen, liver, and kidneys, whereas no sys-

FIG. 4. C3 mediates in vivo control of *B. parapertussis* Δ*wbm*. Groups of three 4- to 6-week-old C3$^{-/-}$, C5$^{-/-}$, or CD11b$^{-/-}$ mice were inoculated with 5×10^5 CFU of *B. parapertussis* (filled bars) or *B. parapertussis* Δ*wbm* (open bars). The number of CFU recovered from the lungs at day 3 postinoculation is expressed as the log$_{10}$ means ± standard errors (error bars). ∗, $P \leq 0.05$. The limit of detection is log$_{10}$1, indicated as the y intercept.

temic colonization by *B. parapertussis* Δ*wbm* was detected (Fig. 5C). Groups of 15 to 20 RAG1$^{-/-}$ mice were inoculated with either *B. parapertussis* or *B. parapertussis* Δ*wbm* and monitored for survival. Mice infected with *B. parapertussis* survived for 2 weeks before showing signs of lethal bordetellosis. By day 17 postinoculation, 50% of the mice had died and all mice succumbed to *B. parapertussis* infection by day 21 postinoculation, consistent with previously reported findings (62). In contrast, all mice inoculated with *B. parapertussis* Δ*wbm* survived until the end of the experiment (day 100) without any overt signs of disease (Fig. 5D and data not shown).
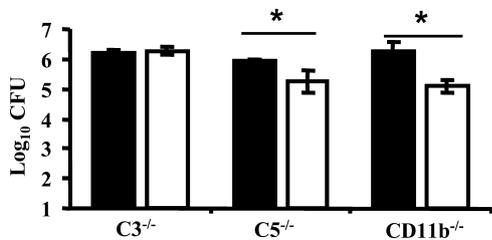
To determine whether complement-mediated control of *B. parapertussis* Δ*wbm* prevented systemic spread and enabled the survival of *B. parapertussis* Δ*wbm*-infected mice, groups of RAG1$^{-/-}$ mice were treated with CVF to deplete complement. These mice were then either sham inoculated or inoculated with *B. parapertussis* Δ*wbm*. CVF-treated, sham-inoculated animals showed no sign of deteriorating disease up to day 35. In contrast, CVF-treated, *B. parapertussis* Δ*wbm*-inoculated animals survived for 2 weeks before showing signs of lethal bordetellosis, a survival curve similar to that of RAG1$^{-/-}$ mice inoculated with *B. parapertussis* (Fig. 4D). By day 21 postinoculation, all CVF-treated, *B. parapertussis* Δ*wbm*-inoculated mice had succumbed to infection (Fig. 4D). Therefore, the
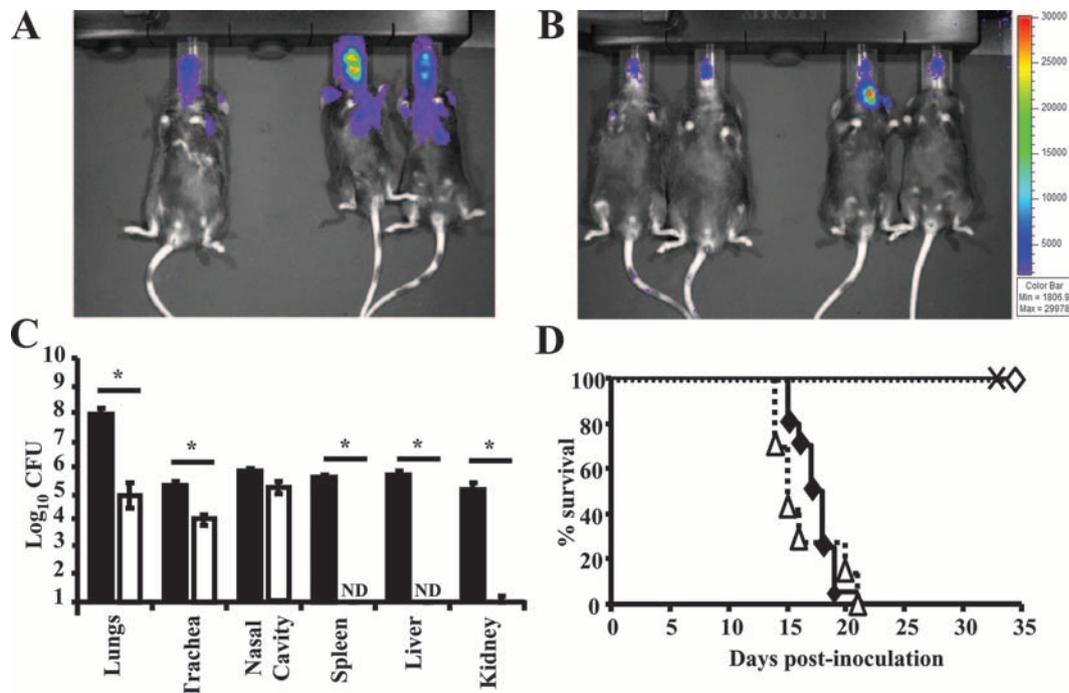
FIG. 5. Role of O antigen in the lethality and systemic spread of *B. parapertussis* infections. RAG1$^{-/-}$ mice were infected with (A) *B. parapertussis*::luciferase or (B) *B. parapertussis* Δ*wbm*::luciferase, and the light production was visualized on day 21 postinoculation and is represented by heat plot. (C) Groups of three to six 4- to 6-week old RAG1$^{-/-}$ mice were inoculated with *B. parapertussis* (closed bars) or *B. parapertussis* Δ*wbm* (open bars) and dissected on day 17 postinoculation for bacterial enumeration in the indicated organs. CFU are expressed as the log$_{10}$ means ± standard errors (error bars). ∗, $P \leq 0.05$. The limit of detection is log$_{10}$1, indicated as the *y* intercept. ND, no CFU were detected in the sample. (D) Groups of 15 to 20 4- to 6-week-old RAG1$^{-/-}$ mice were inoculated with 5×10^5 CFU of *B. parapertussis* (filled symbols) or *B. parapertussis* Δ*wbm* (open symbols) or were sham inoculated (×) with (triangle or ×) or without (diamond) CVF treatment and were monitored for survival.

depletion of complement allowed the systemic spread of *B. parapertussis* Δ*wbm*, further indicating that complement mediates the more efficient control of the O antigen-deficient strain.

DISCUSSION

Here we define a specific role for the O antigen of *B. parapertussis* in a murine model of infection. *B. parapertussis* Δ*wbm* is controlled more efficiently than are the wild-type bacteria during the first week of infection in wild-type mice (Fig. 1). This defect is dependent on the presence of complement component C3 (Fig. 2 and 4) but not neutrophils or macrophages. O antigen prevents C3 deposition on the surface of *B. parapertussis* (Fig. 3) and allows for the systemic spread of *B. parapertussis* in immunodeficient mice (Fig. 5). When complement was depleted, *B. parapertussis* Δ*wbm* was also able to lethally infect immunodeficient mice, indicating that O antigen is not required for any aspect of infection and systemic spread in the absence of complement. Together these data indicate that an important role of O antigen in infection and virulence of naïve hosts by *B. parapertussis* is complement resistance.

Activation of the complement cascade results in several different antimicrobial effects, including opsonization, neutrophil recruitment, scavenger receptor recognition, complement cascade amplification, and formation of the MAC (59). In our study, we found that *B. parapertussis* Δ*wbm* has no observable defect compared to wild-type mice when all of these complement activities were removed (C3$^{-/-}$ or CVF-treated mice), but was defective when individual pathways were eliminated (C5$^{-/-}$, neutrophil-depleted, or CD11b$^{-/-}$ mice) (Fig. 2 and 4). This suggests redundancy in the protective mechanisms of complement against *B. parapertussis*.

B. pertussis utilizes the same host population as does *B. parapertussis* and causes the same disease, although some studies show that *B. parapertussis*-caused symptoms are milder and/or shorter in duration; other studies suggest that this may not be the case (7, 25, 33). However, *B. pertussis* does not express an O antigen, which is apparently not crucial to the infection of human hosts by the bordetellae. As the maintenance of O antigen by *B. parapertussis* confers resistance to complement deposition in vitro and complement-mediated control in vivo, *B. pertussis* likely developed other mechanisms to protect itself against the complement cascade. For example, BrkA is produced by *B. pertussis* and is known to confer resistance to serum-mediated killing (5, 20). In addition, *B. pertussis* is known to bind C4BP and to acquire resistance to complement during growth in vivo by some unknown mechanism (41). While O antigen may not be crucial to *B. pertussis*, the evasion of complement-mediated killing by some mechanism may be vital.

In addition to our observations that O antigen confers resistance to the innate immune function of complement, we have also begun to address the role of *B. parapertussis* O antigen in the evasion of adaptive immune responses. Our

laboratory has recently shown that O antigen prevents *B. pertussis*-induced antibodies from binding to *B. parapertussis*, allowing *B. parapertussis* to evade *B. pertussis*-induced immunity (60). While the inhibition of complement-mediated killing seems to be important to the infection of naïve hosts, the evasion of cross immunity may have enabled *B. parapertussis* to invade populations in which *B. pertussis* was already circulating (8, 60). Importantly, current vaccines have little effect against *B. parapertussis* (18) and it has been suggested that the prevalence of *B. parapertussis* may have increased since the introduction of acellular pertussis vaccines (29). Mechanisms by which *B. parapertussis* avoids host immunity are of mounting importance due to the recent resurgence of whooping cough over the last 2 decades, particularly as the contribution of *B. parapertussis* to this resurgence is unclear (13, 14, 19). An understanding of the protective immune response against *B. parapertussis* is essential to continue to improve whooping cough vaccines.

ACKNOWLEDGMENTS

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