

Original article

Different mechanisms of vaccine-induced and infection-induced immunity to *Bordetella bronchiseptica*

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Abstract

A recent resurgence in the number of cases of whooping cough, and other respiratory diseases caused by members of the bordetellae, in vaccinated populations has demonstrated the need for a thorough understanding of vaccine-induced immunity to facilitate more intelligent vaccine design. In this work, we use a murine model of respiratory infection using the highly successful animal pathogen, *Bordetella bronchiseptica*. Since previously infected animals have been shown to resist re-infection by *B. bronchiseptica*, we sought to examine the differences between vaccine-induced immunity and infection-induced immunity. Both prior infection and vaccination conferred nearly complete protection in the lungs, however, only prior infection resulted in significant protection in the upper respiratory tract. While immunity induced by prior infection offered significant protection even in the absence of complement or FcγRs, vaccination-induced protection required both complement and FcγRs. Although vaccination induced higher titers of *B. bronchiseptica*-specific antibodies, this serum was less effective than infection-induced serum in clearing bacteria from the lower respiratory tract. Together these findings highlight substantial differences between the mechanisms involved in vaccine- and infection-induced protective immunity.

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1. Introduction

The *Bordetella* genus contains three closely related gram-negative bacteria that cause respiratory infections in humans and other mammals [1,2]. *B. pertussis* and *B. parapertussis* infect humans and cause the acute and severe respiratory disease, whooping cough [3–5]. *B. bronchiseptica* typically causes asymptomatic infections in a wide range of non-human mammals and persists long term within its hosts [6–8]. However, it is also associated with respiratory diseases and is the etiological agent of atrophic rhinitis in swine, kennel cough in dogs, and snuffles in rabbits [9].

Many commercial vaccines currently used to protect against *B. bronchiseptica* have been shown to induce high titers of serum antibodies and protect against severe disease [9–11]. However, *B. bronchiseptica* has often been isolated from the nasal cavities of animals in vaccinated populations [6,12] suggesting that vaccines fail to protect animals from infection. In contrast, previously infected swine and guinea pigs have been shown to resist re-infection by *B. bronchiseptica* [13,14], suggesting that infection-induced immunity is more protective than vaccine-induced immunity. Comparing the protective responses involved in both infection- and vaccine-induced immunity may provide clues to understanding the failure of vaccines to prevent subsequent infections.

In the current study we compared protection against *B. bronchiseptica* in vaccinated mice to that observed in mice recovering from a prior infection and show that infection-induced

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and vaccine-induced immunity differ in their mechanisms of protection. We demonstrate that while parenterally administered vaccines confer protection only in the lungs, an anamnestic response due to a previous infection confers protection throughout the respiratory tract. Our data also indicate that, in spite of the higher antibody titers, serum from vaccinated animals is less effective than serum from infected animals in clearing *B. bronchiseptica*. Additionally, vaccine-induced immunity requires Fc γ receptors and complement for efficient bacterial clearance from the lungs, but infection-induced immunity is independent of complement, and more effective than vaccination in the absence of Fc γ receptors. These results may help define the mechanism behind the observed differences between vaccine and infection-induced immunity.

2. Materials and methods

2.1. Mice

C57BL/6 mice and μ MT mice [15] were purchased from The Jackson Laboratory (Bar Harbor, ME, USA). Fc γ R $^{-/-}$ mice were obtained from Taconic (Hudson, NY, USA) and have been described elsewhere [23]. C3 $^{-/-}$ mice have been described elsewhere and were a kind gift from Dr. Rick Wetsel [16]. Mice were maintained in micro-isolator cages under specific pathogen-free conditions, and were treated in accordance with institutional guidelines. Groups of 4 mice (4–6 weeks old) were used for all experiments.

2.2. Bacterial strains and inoculations

B. bronchiseptica maintained on Bordet-Gengou agar (Difco) plates was inoculated into Stainer-Scholte broth at optical density of 0.1 or lower, and grown to mid-log phase at 37 °C on a roller drum. RB50, the wild-type strain of *B. bronchiseptica*, and the O-antigen mutant of RB50 (RB50Δwbm) have been described previously [8]. Mice lightly sedated with isoflurane (IsoFlo-Abbott Laboratories) were intranasally inoculated with *B. bronchiseptica* by pipetting 50 μ l of the inoculum (1×10^7 CFU/ml) onto the tip of the external nares. Mice were sacrificed 3 days post-inoculation and lungs, tracheae, and nasal cavities were homogenized in Phosphate-buffered saline (PBS) for the quantification of bacterial numbers. Dilutions of the homogenate were plated on Bordet-Gengou agar plates containing 20 μ g of streptomycin per ml, and the number of CFUs were determined after 2 days of incubation at 37 °C. For experiments involving the passive transfer of serum, 200 μ l of serum collected from untreated, vaccinated, or convalescent mice was injected intraperitoneally into mice before inoculation with *B. bronchiseptica*. Mice were sacrificed 3 days post-inoculation and bacterial colonization in the various organs was quantified as described above. For lung homogenate passive transfer experiments, lungs from untreated, vaccinated or convalescent mice were excised and homogenized. The lung homogenate was then filtered through a screen with 60 micron-wide pores. The homogenate was then frozen at -80 °C until its use, when 50 μ L was pipetted

onto the external nares of a lightly sedated mouse immediately after they were inoculated with *B. bronchiseptica*.

2.3. Vaccinations and re-infections

Heat-killed bacterial vaccine was prepared by heating bacteria grown to mid-log phase at 80 °C for 30 min. Mice were immunized intraperitoneally twice at 2 week intervals with 10⁸ heat-killed bacteria in 1 ml PBS. The mice were then challenged intranasally with *B. bronchiseptica* two weeks after the second vaccination, as described above. Mice were also immunized with 0.05 piglet dose of two commercial *B. bronchiseptica* vaccines, ProSystem B.P.E. (Intervet) and Borde-Cell (Agri-Labs) (data not shown). For re-infections, the second infection was administered 70 days after the first infection.

2.4. Western blot analysis

Wild-type *B. bronchiseptica* RB50 (wt) and isogenic *B. bronchiseptica* Δwbm (Δwbm) cultures were inoculated into Stainer Scholte broth and grown to mid-log phase as described above. Whole cell extract containing a total of 20 μ g of protein from wt or Δwbm was resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and analyzed by western blotting with vaccine-induced immune serum or with infection-induced immune serum. All lanes were probed with Goat Anti-Mouse Ig(H + L)HRP, Human adsorbed (R & D Systems). The blot was developed with enhanced chemiluminescence (ECL) detection system (Amersham).

2.5. Analysis of antibody response

C57BL/6 mice were either intranasally infected or intraperitoneally vaccinated as described above, and serum was collected on day 28 post-infection or post-vaccination. Titers of anti-*B. bronchiseptica* antibodies were determined by ELISA using polyvalent anti-mouse secondary antibodies as described elsewhere [17]. Specific class and isotype of antibodies were determined using appropriate secondary antibodies (Southern Biotechnology Associates and Pharmingen). End-point antibody titers were expressed as the reciprocal of the dilution giving an OD at 405 nm of 1 standard deviation above that of negative controls after a 30-min incubation.

2.6. Statistical analysis

All experiments were repeated at least twice with similar results. All data points were analyzed for statistical significance by unpaired Student's *t*-test. Statistical significance was assigned when *P* values were less than 0.05.

3. Results

3.1. Vaccine-induced immunity against *B. bronchiseptica* differs from infection-induced immunity

In order to compare protection conferred by vaccination to that of prior infection, mice were either intraperitoneally

vaccinated twice with 10^8 CFU heat-killed *B. bronchiseptica* at two-week intervals, or were allowed to recover from a previous *B. bronchiseptica* infection before bacterial challenge. As previously shown with this high dose inoculation regimen (5×10^5 CFU in 50 µl PBS intranasally), naïve mice had more than 10^6 CFU in the nasal cavity and lungs, and 10^3 CFU in the trachea, on day 3 post-inoculation [8, and Fig. 1]. Mice that were convalescent from a previous *B. bronchiseptica* infection completely cleared the subsequent infection from the trachea and lungs, and had 1000-fold fewer bacteria in the nasal cavity than naïve animals. In contrast, mice that were vaccinated cleared bacteria from the lungs, but harbored 10^6 bacteria in the nasal cavity (Fig. 1). The apparent protection observed in the trachea after vaccination was not statistically significant. These data indicate that both vaccine- and infection-induced immunity provide protection in the lungs, but only infection-induced immunity confers protection in the trachea and the nasal cavity. We also studied protection using 0.05 piglet dose of two commercial *B. bronchiseptica* vaccines, ProSystem B.P.E. (Intervet) and Borde-Cell (Agri-Labs), and obtained similar results (data not shown).

3.2. *B* cells are required for vaccine-induced immunity to *B. bronchiseptica*

We next sought to investigate if the difference between protection conferred by infection and vaccination was due to different mechanisms of bacterial clearance. Since we have previously shown that *B* cells are required for clearance of *B. bronchiseptica* from the respiratory tracts of mice [18], we examined the role of *B* cells in vaccine-mediated protection against *B. bronchiseptica*, using *B* cell deficient (μ MT) mice [15]. Naive or vaccinated μ MT mice were challenged with *B. bronchiseptica*, as described above, and bacterial numbers in

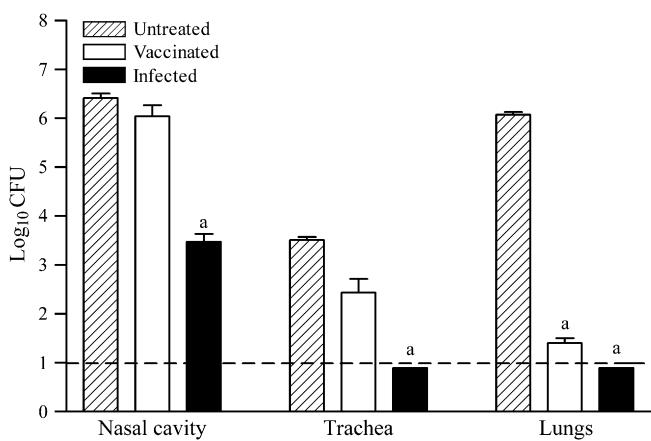


Fig. 1. Comparison of vaccine-induced and infection-induced clearance of *B. bronchiseptica*. C57BL/6 mice that were unvaccinated, vaccinated twice with 10^8 heat-killed *B. bronchiseptica*, or previously infected were intranasally challenged with 5×10^5 CFU of *B. bronchiseptica* in a 50 µl volume of phosphate buffer saline (PBS). Groups of four mice were sacrificed to determine bacterial colonization in the nasal cavity, trachea and lungs 3 days post-challenge. Data points are presented as mean \log_{10} CFU/organ and standard error. ^a $P < 0.05$ when compared with corresponding untreated group. The dashed line represents the lower limit of detection.

respiratory organs were determined 3 days post-inoculation (Fig. 2). *B. bronchiseptica* colonization levels 3 days post-inoculation in naïve μ MT mice were similar to that of naïve C57BL/6 mice (Fig. 1). However, unlike C57BL/6 mice, vaccination of μ MT mice did not affect bacterial numbers in the lungs (Fig. 2). The failure of vaccination to protect μ MT mice indicates that *B* cells are required and that other aspects of immunity, including T cells, are known to contribute [19,20] but appear to not be sufficient to provide substantial protection against *B. bronchiseptica* in the respiratory tract.

3.3. Vaccine-induced antibodies are less effective than infection-induced antibodies in clearance of *B. bronchiseptica* from the lower respiratory tract

We next sought to investigate if antibodies alone were sufficient to confer vaccine-induced and infection-induced protection against *B. bronchiseptica*. Serum was collected from mice 28 days after infection (infection-induced serum) or 28 days after the initial vaccination (vaccine-induced serum). We first compared the antibody titers of the two sera by ELISA and found that serum from vaccinated animals contained titers ranging from 10-fold (IgM) to 100-fold (IgG3) higher than infection-induced serum (Fig. 4A). Interestingly, IgA, which was induced by infection, was not detected (titer < 50) in vaccine-induced serum. Additionally, titers of polyclonal *B. bronchiseptica*-specific antibodies were similar on day 28 in the lungs of vaccinated and convalescent mice (data not shown). While IgA was undetectable in the lungs of vaccinated mice (titer < 50), it was found at high titers (>200) in the lungs of mice that had been previously infected.

We have previously shown that serum antibodies from a convalescent animal infected with *B. bronchiseptica*

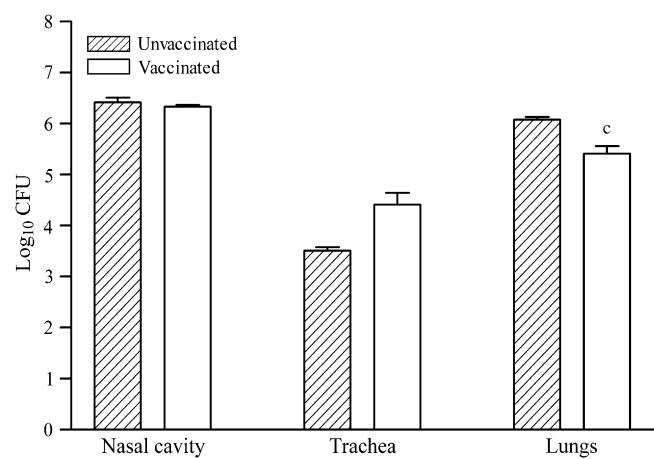


Fig. 2. Vaccine-induced clearance of *B. bronchiseptica* from the respiratory tract by μ MT mice. Unvaccinated μ MT mice or μ MT mice vaccinated twice with 10^8 heat-killed *B. bronchiseptica* were intranasally challenged with 5×10^5 CFU of *B. bronchiseptica* in a 50 µl volume, 2 weeks after the second vaccination. Groups of four mice were sacrificed to determine bacterial colonization in the nasal cavity, trachea and lungs 3 days post-challenge. Data points are presented as mean \log_{10} CFU/organ and standard error. ^c $P < 0.05$ when compared with corresponding wild-type group (Fig. 1).

effectively cleared bacteria from the lower respiratory tract [18]. In this study, we compared the ability of passively transferred serum antibodies collected from vaccinated and infected mice to reduce the numbers of *B. bronchiseptica* in various respiratory organs (Fig. 3A). As shown previously [18], passive transfer of 0.2 ml of infection-induced serum brought about a 100,000-fold reduction in bacterial numbers in the lungs and completely cleared the infection from the trachea within three days, but did not affect bacterial numbers in the nasal cavity. However, passive transfer of 0.2 ml of vaccine-induced serum brought about only a 1000-fold reduction in bacterial numbers in the lungs, and no significant reduction in numbers in the trachea or the nasal cavity of C57BL/6 mice (Fig. 3A). Immunoglobulin isotype A is an important aspect of mucosal immunity and relatively high levels are induced in the lungs

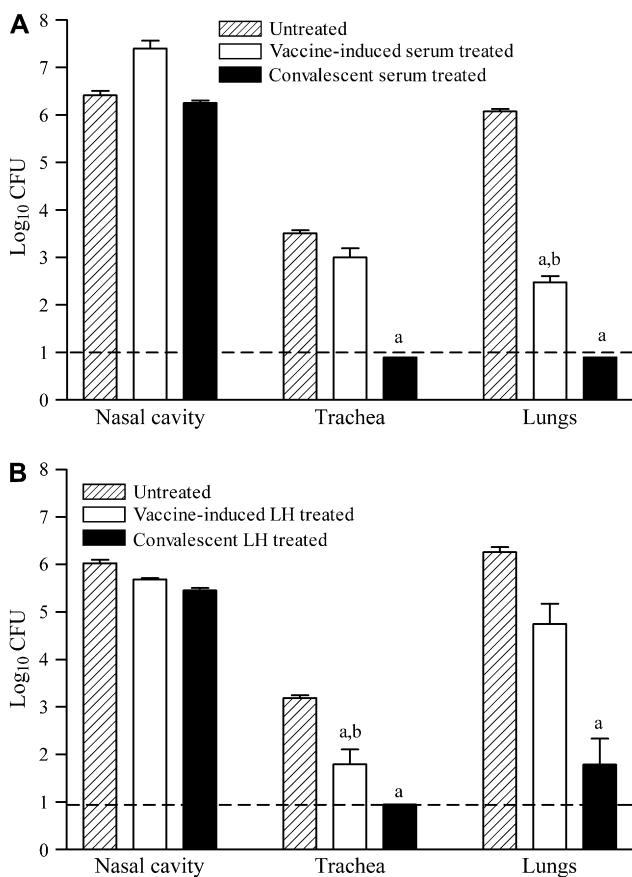


Fig. 3. Clearance of *B. bronchiseptica* from the respiratory tract by passive transfer of serum or lung homogenate from vaccinated or convalescent mice. (A) C57BL/6 mice were passively immunized with either serum from convalescent mice (infection-induced) or serum from vaccinated mice (vaccine-induced) and subsequently challenged intranasally with 5×10^5 CFU *B. bronchiseptica* in a 50 μ l volume. (B) C57BL/6 mice were inoculated with 5×10^5 CFU *B. bronchiseptica* in a 50 μ l volume. Subsequently, the mice received 50 μ l of lung homogenate (LH) intranasally from naïve, vaccinated, or convalescent mice. Untreated non-immunized mice infected with *B. bronchiseptica* acted as controls. Groups of four mice were sacrificed to determine bacterial colonization in the nasal cavity, trachea and lungs 3 days post-challenge. Data points are presented as mean \log_{10} CFU/organ and standard error. ^a $P < 0.05$ when compared with corresponding untreated group. ^b $P < 0.05$ when compared with corresponding convalescent serum/LH treated group. The dashed line represents the lower limit of detection.

upon a respiratory infection, prompting us to compare the effects of passively transferring lung homogenate from naïve, vaccinated, and convalescent mice intranasally at the time of infection. An intranasal transfer of lung homogenate from a naïve mouse had no effect on bacterial numbers throughout the respiratory tract. Interestingly, transferring lung homogenate from a previously infected mouse caused a 10,000-fold decrease in CFU in the lungs while lung homogenate from a vaccinated mouse had a modest effect that was statistically insignificant (Fig. 3B). Lung homogenate from an infected mouse was also more effective in reducing bacterial numbers in the trachea (Fig. 3B). These data suggest that, in spite of the higher antibody titers, vaccine-induced antibodies are less effective than infection-induced antibodies in protecting against a *B. bronchiseptica* infection. Additionally, the enhanced clearance throughout the respiratory tract observed in convalescent mice (relative to vaccinated mice) may be mediated by IgA as this was the only isotype that was at higher titers in infection-induced serum than vaccine-induced serum (Figs. 1 and 4A). IgA was also detected at significant titers in infection-induced lung homogenate, but was undetectable in vaccine-induced lung homogenate (data not shown).

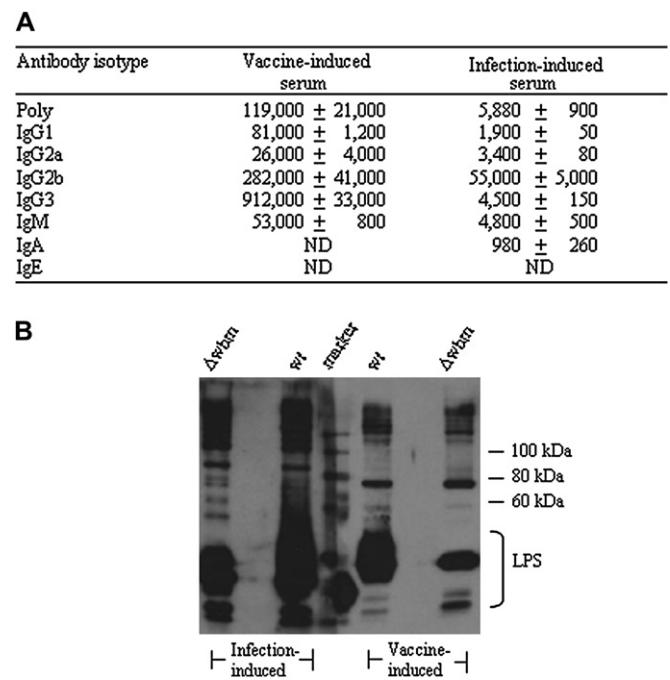


Fig. 4. Comparison of antibody titers and antigen recognition profile for infection-induced and vaccine-induced sera. (A) *B. bronchiseptica* specific titers for different isotypes of antibodies as measured by enzyme-linked immunosorbent assay were compared in pooled serum samples collected 28 days after vaccination with 10^8 heat-killed *B. bronchiseptica* (vaccine-induced serum) or 28 days after intranasal infection with 5×10^5 CFU of *B. bronchiseptica* (infection-induced serum). Values represent the mean ± the standard error of the Ig titers detected. ND, not detected. (B) Pooled serum samples were collected from C57BL/6 mice that were either vaccinated with heat-killed *B. bronchiseptica* or were convalescent from a previous *B. bronchiseptica* infection. Whole cell extracts from *B. bronchiseptica* RB50 (wt) and isogenic *B. bronchiseptica* Δ wbtm (Δ wbtm) cultures were probed with infection-induced and vaccine-induced serum by Western blot analysis.

To investigate if this may be due in part to differential antigen recognition by the two sera, we performed a western blot analysis by probing whole cell extracts of wild-type *B. bronchiseptica* RB50 (wt) and isogenic *B. bronchiseptica* that lacks the O-antigen (Δ wbm) with infection-induced and vaccine-induced serum (Fig. 4B). Vaccine-induced serum recognized a smaller number of antigens that included LPS, an antigen of around 70 kDa, and a range of high molecular weight (>100 kDa) antigens. Infection-induced serum, on the other hand, recognized a larger number of molecules across the range of molecular weights.

3.4. Complement is required for efficient vaccine-induced, but not infection-induced, clearance of *B. bronchiseptica*

The differences in the protective abilities of vaccine-induced and infection-induced antibodies suggest that bacterial clearance may occur by different mechanisms, potentially involving different antibody-effector functions. These may involve neutralization, activation of the complement system or opsonization of bacteria. Activated complement components can mediate bacterial killing by direct bacterial lysis via formation of the membrane attack complex (MAC), by opsonization or by recruitment and activation of inflammatory cells [21]. We investigated the requirement for complement in protection against *B. bronchiseptica* using mice that lack complement component C3 (C3^{-/-} mice), and hence lack the major effector actions of complement [16,22]. C3^{-/-} mice that were either vaccinated with heat-killed bacteria or were convalescent from previous infection were challenged with *B. bronchiseptica*, as described above, and bacterial numbers in the respiratory organs were determined 3 days post-challenge (Fig. 5). *B. bronchiseptica* colonization levels in naïve C3^{-/-} mice were indistinguishable from naïve C57BL/6 mice on day 3 post-infection and the protection seen in previously infected C3^{-/-} mice was as great as that in C57BL/6 mice (Fig. 1). However, compared to the 10⁶-fold effect in wild type mice, vaccination of C3^{-/-} mice resulted in only a 100-fold reduction in bacterial numbers in the lungs by day 3 post-challenge. Thus, in contrast to infection-induced protection, vaccine-induced protection requires complement for efficient clearance of *B. bronchiseptica* from the lungs.

3.5. Fc γ receptors are required for efficient vaccine-induced, but not infection-induced, clearance of *B. bronchiseptica*

An activated complement cascade generates anaphylatoxins such as C3a and C5a which induce local inflammatory responses and recruit phagocytic cells to the site of infection. Phagocytic cells bearing Fc receptors or complement receptors play a crucial role in engulfment of opsonized bacteria [23,24]. Bacterial clearance mediated by Fc receptors therefore acts in conjunction with complement in antibody-mediated protection. We used mice lacking Fc γ receptors (Fc γ R^{-/-}) to assess the requirement for Fc γ R-mediated phagocytosis in protection

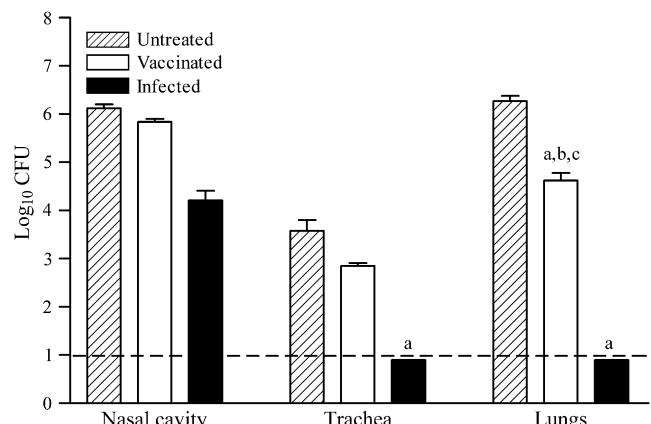


Fig. 5. Vaccine-induced and infection-induced clearance of *B. bronchiseptica* from the respiratory tract by C3^{-/-} mice. Unvaccinated C3^{-/-} mice, C3^{-/-} mice vaccinated twice with 10⁸ heat-killed *B. bronchiseptica*, or C3^{-/-} mice convalescent from a previous *B. bronchiseptica* infection were intranasally challenged with 5 × 10⁵ CFU *B. bronchiseptica* in a 50 µl volume. Groups of four mice were sacrificed to determine bacterial colonization in the nasal cavity, trachea and lungs 3 days post-challenge. Data points are presented as mean log₁₀ CFU/organ and standard error. ^aP < 0.05 when compared with corresponding untreated group. ^bP < 0.05 when compared with corresponding infected group. ^cP < 0.05 when compared with corresponding wild-type group (Fig. 1). The dashed line represents the lower limit of detection.

against *B. bronchiseptica*. Fc γ R^{-/-} mice were either vaccinated with heat-killed *B. bronchiseptica*, or were convalescent from previous *B. bronchiseptica* infection. Due to the possibility of a reduced LD₅₀ in Fc γ R^{-/-} mice that is being addressed separately (G.S. Kirimaneeswara and E.T. Harvill, unpublished data), we used a low dose low volume inoculation regimen of 100 bacteria in 5 µl of PBS for the initial infection. These mice, or naïve Fc γ R^{-/-} mice were challenged with *B. bronchiseptica*, and bacterial numbers in the respiratory organs were determined 3 days post-challenge, as described above (Fig. 6). *B. bronchiseptica* colonization levels in naïve Fc γ R^{-/-} mice were similar to naïve C57BL/6 mice on day 3 post-challenge. Infection and vaccine-induced protection seen in the trachea and the nasal cavity of Fc γ R^{-/-} mice was similar to that of C57BL/6 mice (Fig. 1). However, unlike vaccinated or previously infected C57BL/6 mice which cleared the infection from the lungs, Fc γ R^{-/-} mice were less efficient and brought about a 1000-fold (vaccinated) or 10⁵-fold (previously infected) reduction in bacterial numbers by day 3 post-challenge (Fig. 6). Fc γ R^{-/-} mice that were previously infected had significantly lower bacterial numbers in the lungs than those that were vaccinated, indicating that vaccination generates less effective immunity than prior infection in these mice. Thus, Fc γ Rs are required for efficient vaccine-induced, and contribute to infection-induced protection against *B. bronchiseptica*.

4. Discussion

The continued prevalence of *B. bronchiseptica* in vaccinated hosts suggests that *B. bronchiseptica* vaccines control disease, but are unable to prevent subsequent infections [6,12]. Interestingly, studies in mouse models have shown

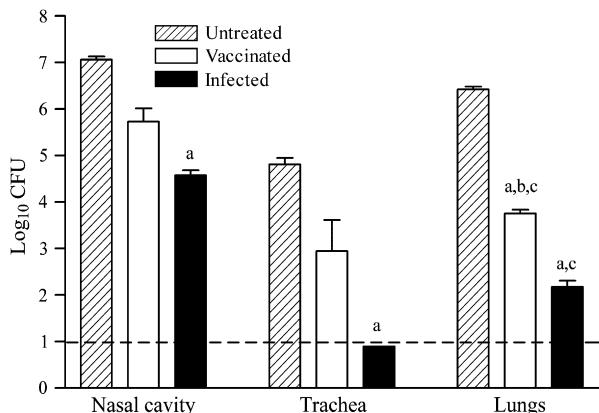


Fig. 6. Vaccine-induced and infection-induced clearance of *B. bronchiseptica* from the respiratory tract by Fc γ R $^{-/-}$ mice. Unvaccinated Fc γ R $^{-/-}$ mice, Fc γ R $^{-/-}$ mice vaccinated twice with 10^8 heat-killed *B. bronchiseptica*, or Fc γ R $^{-/-}$ mice convalescent from a previous *B. bronchiseptica* infection were intranasally challenged with 5×10^5 CFU of *B. bronchiseptica* in a 50 μ l volume. Groups of four mice were sacrificed to determine bacterial colonization in the nasal cavity, trachea and lungs 3 days post-challenge. Data points are presented as mean log₁₀ CFU/organ and standard error. ^a*P* < 0.05 when compared with corresponding untreated group. ^b*P* < 0.05 when compared with corresponding infected group. ^c*P* < 0.05 when compared with corresponding wild-type group (Fig. 1). The dashed line represents the lower limit of detection.

that a previous infection with *B. bronchiseptica* induces an immune response that can rapidly control a subsequent infection [13,14, Fig. 1, and L. Gopinathan. and E.T. Harvill, unpublished data], suggesting that infection-induced anamnestic protection is more effective than vaccine-induced protection. The difference in the level of protection conferred by vaccination versus infection suggests that immunity induced by these strategies may be mechanistically different. Here we show differences in these mechanisms that may explain clinical findings regarding incidence of *Bordetella* infections in vaccinated populations.

Prior infection with *B. bronchiseptica* conferred protection against a subsequent infection in all the respiratory organs. Vaccination was effective in reducing bacterial burden in the lungs, but not in the trachea and nasal cavity. Both vaccine- and infection-induced protection required neutrophils and macrophages for efficient clearance of *B. bronchiseptica* infection (data not shown). Vaccine-induced, but not infection-induced protection required complement. Fc γ receptors were also required for vaccine-induced protection. The 100s of CFU in the lungs of previously infected Fc γ R $^{-/-}$ mice suggest that Fc γ Rs may also contribute to infection-induced immunity, suggesting differences in the mechanisms of immunity generated by infection and vaccination. Since both infection and vaccination induced immunity required B cells, we investigated the protection conferred by antibodies generated by these immunizations. Interestingly, passive transfer of serum revealed that, despite higher titers, vaccine-induced serum was less effective than infection-induced serum in protecting against *B. bronchiseptica* infection in the lungs. Similar effects were observed upon passive transfers of lung homogenate from vaccinated versus convalescent mice. ELISA data

indicated that the two sera differ in that anti-*B. bronchiseptica* titers were higher in vaccine-induced serum for all isotypes except IgA, which was absent from vaccine-induced serum. Similar polyclonal titers were observed in vaccine- and infection-induced lung homogenate, but IgA was absent from vaccine-induced lung homogenate. Furthermore, the antigen recognition patterns were different between the two sera, with vaccine-induced serum recognizing a smaller number of antigens than infection-induced serum. It will be interesting to determine the dominant isotypes involved in immunity to *Bordetella* and the specific antigens recognized by these two sera.

The strong serum antibody response induced by vaccination and the requirement for both Fc γ Rs and complement suggests that vaccine-induced protection is primarily mediated by serum antibodies. This is supported by other studies from our laboratory that show passively transferred serum antibodies similarly require Fc γ Rs and complement for bacterial clearance [24,25]. On the other hand, infection-induced protection is independent of complement, suggesting that protection is mediated by other immune mechanisms along with serum antibodies. Of particular importance is the generation of IgA, a dominant isotype of the mucosal immune system [26]. Although vaccination does not effectively induce mucosal immunity, intranasal vaccination has been shown to elicit antigen-specific immune responses in both the mucosal and the systemic compartments [27–29]. A comparative analysis of mucosal immunity, particularly the role of IgA elicited by infection, systemic vaccination, and intranasal vaccination with *B. bronchiseptica* could provide an explanation for the different mechanisms of infection and vaccine-induced protection. We are currently trying to understand the role of IgA in vaccine and infection-induced immunity using IgA $^{-/-}$ mice.

Analyses such as those presented in this study may be helpful in understanding the reasons for the failures of *Bordetella* vaccines and will aid in designing improved vaccines that can effectively induce protection throughout the respiratory tract, and thus prevent both disease and infection. The circulation of *Bordetella pertussis* within vaccinated populations and the recent resurgence of *B. pertussis*-associated disease underscore the failure of current vaccines to protect against subclinical infections and to provide lifelong immunity [30]. We have examined the generation and function of the immune response to *B. bronchiseptica*, a closely related subspecies that naturally infects laboratory animals, providing a natural host-pathogen interaction model to study protective immunity to *Bordetella*. These results are likely to be directly relevant to the many *B. bronchiseptica* vaccines currently in use in animals, which include multiple different live-attenuated mucosal vaccines as well as numerous formulations of injected killed bacteria. They are also likely to be relevant to the many human infections by *B. bronchiseptica*, and possibly to those by *B. pertussis* and/or *B. parapertussis*. In particular, the observation that induction of mucosal immunity may be crucial to preventing subsequent infections could have important implications to the prevention and treatment of this and other respiratory diseases.

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