INFECTION AND IMMUNITY, Sept. 2007, p. 4416–4422 Vol. 75, No. 9
0019-9567/07/$08.00+0 doi:10.1128/IAI.00412-07

Comparative Role of Immunoglobulin A in Protective Immunity against the Bordetellae▽

Daniel N. Wolfe,[1]† Girish S. Kirimanjeswara,[1]†‡ Elizabeth M. Goebel,[2] and Eric T. Harvill[1]*

Department of Veterinary and Biomedical Sciences[1] and The Huck Institutes of the Life Sciences,[2] The Pennsylvania State University, 115 Henning Building, University Park, Pennsylvania 16802

Received 20 March 2007/Returned for modification 19 April 2007/Accepted 13 June 2007

The genus *Bordetella* includes a group of closely related mammalian pathogens that cause a variety of respiratory diseases in a long list of animals (*B. bronchiseptica*) and whooping cough in humans (*B. pertussis* and *B. parapertussis*). While past research has examined how these pathogens are eliminated from the lower respiratory tract, the host factors that control and/or clear the bordetellae from the upper respiratory tract remain unclear. We hypothesized that immunoglobulin A (IgA), the predominant mucosal antibody isotype, would have a protective role against these mucosal pathogens. IgA$^{-/-}$ mice were indistinguishable from wild-type mice in their control and clearance of *B. pertussis* or *B. parapertussis*, suggesting that IgA is not crucial to immunity to these organisms. However, naïve and convalescent IgA$^{-/-}$ mice were defective in reducing the numbers of *B. bronchiseptica* in the upper respiratory tract compared to wild-type controls. Passively transferred serum from convalescent IgA$^{-/-}$ mice was not as effective as serum from convalescent wild-type mice in clearing this pathogen from the tracheae of naive recipient mice. IgA induced by *B. bronchiseptica* infection predominantly recognized lipopolysaccharide-containing O-antigen, and antibodies against O-antigen were important to bacterial clearance from the trachea. Since an IgA response contributes to the control of *B. bronchiseptica* infection of the upper respiratory tract, immunization strategies aimed at inducing *B. bronchiseptica*-specific IgA may be beneficial to preventing the spread of this bacterium among domestic animal populations.

Clinical and experimental findings show that individuals deficient in the immunoglobulin A (IgA) isotype of antibodies, the main immunoglobulin in mucus secretions, are more susceptible to certain sinopulmonary infections (17, 34, 36). In animal studies, IgA is important to immunity against gram-positive bacteria, such as *Streptococcus pneumoniae* (19, 39). These antibodies also effectively reduced the nasal carriage of influenza virus in a mouse model (34). However, there are conflicting reports regarding the contribution of IgA to immunity against gram-negative bacterial pathogens (1, 17, 18, 22).

This study analyzes the protective role of IgA in response to infection by the gram-negative mammalian respiratory pathogens *Bordetella bronchiseptica*, *Bordetella pertussis*, and *Bordetella parapertussis*. *B. bronchiseptica* infects a wide range of mammals and is an etiologic agent of kennel cough in dogs, atrophic rhinitis in pigs, and snuffles in rabbits (4, 23). *B. pertussis* and *B. parapertussis* are human-adapted pathogens that colonize the respiratory tract, causing whooping cough (6, 25). Since these three closely related bacterial species colonize the respiratory mucosa, we hypothesized that *Bordetella*-specific IgA would be important to their elimination from the respiratory tract.

Previously, we showed that B cells are required for clearance of *B. bronchiseptica*, *B. pertussis*, and *B. parapertussis* from the murine respiratory tract, but passively transferred serum antibodies clear these pathogens only from the lower respiratory tract (20, 21, 42). Interestingly, passively transferred serum antibodies have little, if any, effect on these species in the upper respiratory tract (20, 21, 42). Since the passively transferred serum contained low levels of IgA (20, 42), we hypothesized that IgA may be important in controlling these bacteria in the upper respiratory tract.

Here we demonstrate that IgA is essential for controlling *B. bronchiseptica* in the upper respiratory tract of both naïve and immunized hosts. Additionally, a passive transfer of serum from convalescent wild-type mice reduces *B. bronchiseptica* numbers in the trachea more effectively than serum from convalescent IgA$^{-/-}$ mice. We also show that the predominant antigen recognized by IgA induced upon *B. bronchiseptica* infection is O-antigen, a protective antigen for some gram-negative bacteria (31, 35). Antibodies against O-antigen also contributed to bacterial clearance from the trachea. Although *B. bronchiseptica* is very closely related to the human pathogens *B. pertussis* and *B. parapertussis*, our data suggest that IgA may not be important to protection against these bacteria. While *B. pertussis* does not express an O-antigen, *B. parapertussis* does express this virulence factor and yet does not appear to be substantially affected by the presence or absence of IgA.

MATERIALS AND METHODS

Bacterial strains and growth. *B. bronchiseptica* strain RB50 (7), *B. pertussis* strain 536 (33), and *B. parapertussis* strain 12822 (15) have previously been described. RB50G, Tohama1G, and 12822G are gentamicin-resistant derivatives of these strains and have previously been described (12, 42). *B. bronchiseptica* strain RB50Δ*wbm* is an isogenic mutant of RB50 that lacks the genes necessary

* Corresponding author. Mailing address: Department of Veterinary and Biomedical Sciences, The Pennsylvania State University, 115 Henning Building, University Park, PA 16802. Phone: (814) 863-8522. Fax: (814) 863-6140. E-mail: harvill@psu.edu.

† D.N.W. and G.S.K. contributed equally to this work.

‡ Present address: Albany Medical Center, 43 New Scotland Avenue, Albany, NY 12208.

▽ Published ahead of print on 25 June 2007.

for O-antigen synthesis and has been described previously (32). Bacterial strains were maintained on Bordet-Gengou agar (Difco, Albany, NY) supplemented with 10% defibrinated sheep's blood (Hema Resources, OR) and 20 μg/ml streptomycin or 20 μg/ml gentamicin.

Mouse strains, inoculations, and passive transfers. C57BL/6 mice were obtained from Jackson Laboratories, and $IgA^{-/-}$ mice were a kind gift from Innocent Mbawuike, Baylor College of Medicine, Houston, TX, and have been described elsewhere (13, 27). For inoculations, bacteria were grown in Stainer-Scholte broth with supplements to mid-log phase (optical densities of approximately 0.3 at 600 nm) at 37°C on a roller drum and diluted to approximately 10^7 CFU/ml. Four- to 6-week-old mice were lightly sedated with 5% isoflurane (IsoFlo; Abbott Laboratories) in oxygen, and 5×10^5 CFU in 50 μl of phosphate-buffered saline were pipetted onto the tip of the external nares. Convalescent mice were generated by inoculating naïve mice and allowing them to recover for at least 28 days. For passive transfer experiments, convalescent mice were bled by cardiac puncture, the serum layer was isolated, and sera were kept at −80°C until use. A 200-μl aliquot of convalescent-phase serum was injected intraperitoneally into naïve mice, immediately followed by inoculation as described above. Mice were maintained in our *Bordetella*-free breeding facilities at The Pennsylvania State University, and all protocols were reviewed and approved by the university IACUC program.

Harvesting of organs and enumeration of bacteria. For time course experiments, groups of four animals were sacrificed on days 0, 7, 14, 28, 49, 70, or 105 postinoculation and lungs, tracheae, and nasal cavities were collected. Bacterial colonization was determined by homogenization and serial dilution of the indicated organs in phosphate-buffered saline and plating aliquots onto Bordet-Gengou agar with the appropriate antibiotics. Colonies were counted after 2 (for *B. bronchiseptica*) or 4 (for *B. pertussis* and *B. parapertussis*) days of incubation at 37°C.

Rechallenge protocol. For rechallenge experiments, mice were inoculated with 5×10^5 CFU of streptomycin-resistant bacteria as described above. These mice were treated with gentamicin via drinking water (10 mg/ml) for 7 days starting on day 21 postinoculation. On day 28 postinoculation, gentamicin treatment was stopped. On day 30 postinoculation, mice were rechallenged with 5×10^5 CFU of the gentamicin-resistant bacterial strains. These mice were sacrificed at the indicated days post-secondary inoculation for the quantification of both streptomycin-resistant and gentamicin-resistant bacteria.

ELISAs and Western blot assays. Titers of anti-*B. bronchiseptica* antibodies in convalescent-phase sera were determined by enzyme-linked immunosorbent assay (ELISA) using polyvalent anti-mouse secondary antibodies as described previously (2). Specific classes and isotypes of antibodies were determined by using appropriate secondary antibodies (Southern Biotechnology Associates, Birmingham, AL, and Pharmingen, San Diego, CA). For the analysis of antigens that were recognized by IgA, Westerns blot assays were performed on lysates of *B. bronchiseptica* strain RB50 and the isogenic mutant strain lacking O-antigen, RB50Δ*wbm* (32). Lysates were run on a 7% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel under denaturing conditions. Membranes were probed with primary antibodies from pooled *B. bronchiseptica*-induced convalescent-phase serum at a 1:1,000 dilution or pooled lung homogenate from three wild-type mice infected with *B. bronchiseptica* 28 days postinoculation at a 1:50 dilution. Detector antibodies were either polyclonal goat anti-mouse (H+L)–horseradish peroxidase conjugated (Southern Biotechnology, Birmingham, AL) or IgA-specific goat anti-mouse IgA–horseradish peroxidase conjugated (Southern Biotechnology, Birmingham, AL). The membrane was visualized with ECL Western blotting detection reagents (Amersham Biosciences, Piscataway, NJ).

Statistics. Three to four mice were used per group for each experiment, and each experiment was performed at least twice with similar results. All quantified values are expressed as the mean ± standard error (error bars). For comparing bacterial numbers or antibody titers between groups, Student's unpaired *t* tests were used. Differences were assigned statistical significance when the *P* value was <0.05.

RESULTS

IgA is required for reducing *B. bronchiseptica* but not *B. pertussis* or *B. parapertussis* numbers in the upper respiratory tract. We have previously shown that B-cell-deficient mice fail to clear the bordetellae from the respiratory tract, suggesting that antibodies are critical for bacterial clearance (20). Passive transfer of serum antibodies eliminates *B. bronchiseptica*, *B. pertussis*, and *B. parapertussis* from the lungs and tracheae but not nasal cavities (20, 21, 42) (data not shown), suggesting that local antibodies such as IgA may play an important role in the reduction of bacterial numbers in the upper respiratory tract. To address this possibility, groups of four wild-type and $IgA^{-/-}$ mice were intranasally inoculated with *B. bronchiseptica*, *B. pertussis*, or *B. parapertussis*. Mice were sacrificed on days 0, 7, 14, 28, 49, 70, or 105 postinoculation, and the respiratory organs were collected to quantify the numbers of bacteria in these organs. Numbers of *B. bronchiseptica* in the lungs of wild-type and $IgA^{-/-}$ mice were similar, indicating that IgA is not essential for reducing the bacterial load in the lungs (Fig. 1A). Approximately 10- to 100-fold-lower numbers of *B. bronchiseptica* were found in the tracheae of wild-type mice compared to $IgA^{-/-}$ mice at days 14, 28, and 49 postinoculation (Fig. 1B). Furthermore, there were approximately 100-fold fewer bacteria in the nasal cavities of wild-type mice compared to $IgA^{-/-}$ mice from day 14 until the end of our time course (day 105 postinoculation) (Fig. 1C). These results indicate that IgA is important to the reduction of *B. bronchiseptica* numbers in the trachea and nasal cavity but not the lungs.

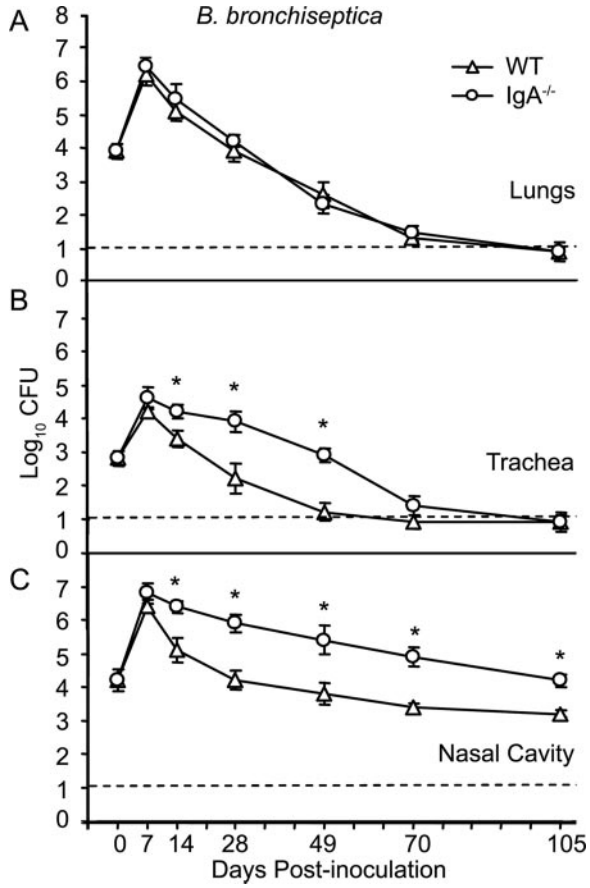

FIG. 1. *B. bronchiseptica* colonization of wild type (WT) versus $IgA^{-/-}$ mice. Groups of four C57BL/6 and $IgA^{-/-}$ mice were inoculated with 5×10^5 CFU of *B. bronchiseptica*. Lungs (A), tracheae (B), and nasal cavities (C) were harvested on days 0, 7, 14, 28, 49, 70, and 105 postinoculation, and the numbers of bacteria were quantified in each organ. Bacterial numbers are expressed as the mean $\log_{10}$ CFU ± the standard error (error bars). The dashed line represents the lower limit of detection.

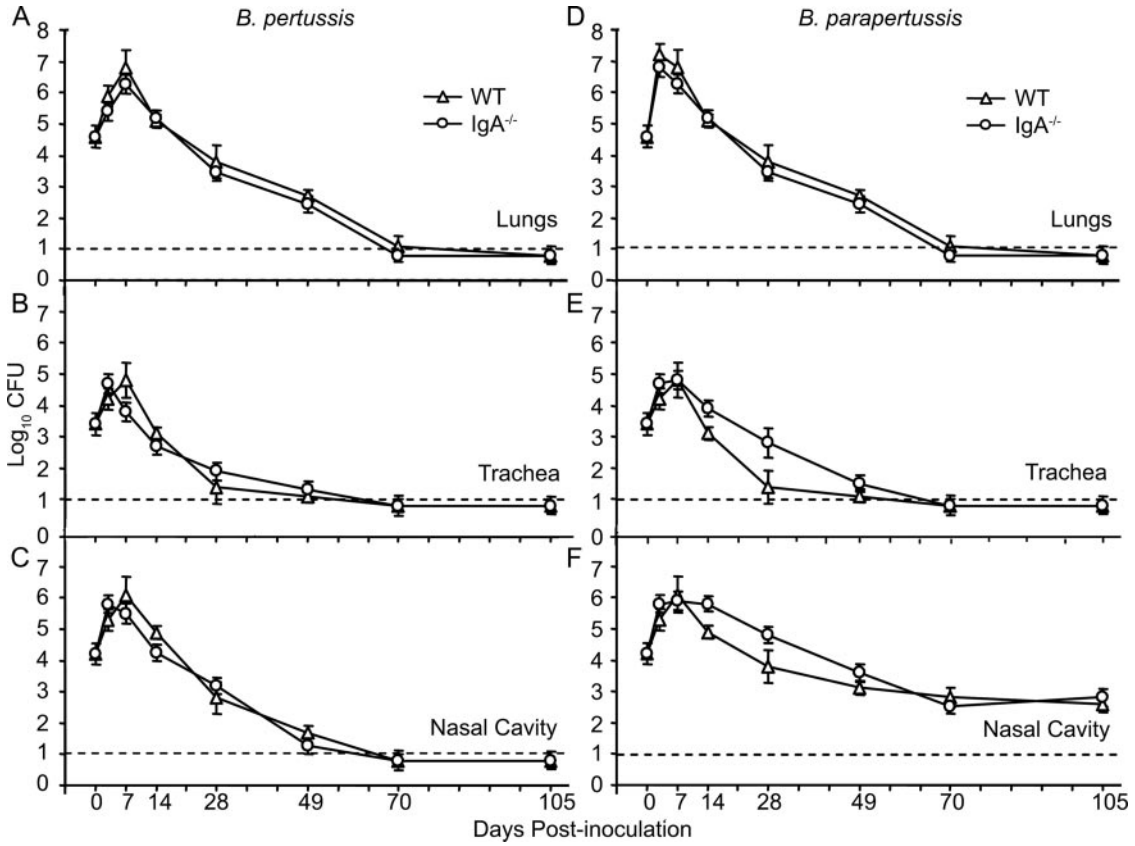

FIG. 2. *B. pertussis* and *B. parapertussis* colonization of wild-type (WT) versus IgA$^{-/-}$ mice. Groups of four C57BL/6 and IgA$^{-/-}$ mice were inoculated with 5×10^5 CFU of *B. pertussis* (A to C) or *B. parapertussis* (D to F). Lungs (A and D), tracheae (B and E), and nasal cavities (C and F) were harvested on days 0, 7, 14, 28, 49, 70, and 105 postinoculation, and the numbers of bacteria were quantified. Bacterial numbers are expressed as the mean $\log_{10}$ CFU ± the standard error (error bars). The dashed line represents the lower limit of detection.

Interestingly, numbers of *B. pertussis* were similar in wild-type and IgA$^{-/-}$ mice throughout the respiratory tract (Fig. 2A to C). Although *B. parapertussis* numbers appeared to be higher in the tracheae and nasal cavities of IgA$^{-/-}$ mice compared to wild-type mice at some time points, there were no significant differences between wild-type and IgA$^{-/-}$ mice throughout the time course (Fig. 2D to F). Together, these data indicate that IgA is not important to clearing *B. pertussis* or *B. parapertussis* from the murine host.

IgA is critical to protection against subsequent *B. bronchiseptica* but not *B. pertussis* or *B. parapertussis* infections. In order to control the spread of *B. bronchiseptica*, sterilizing immunity in the upper respiratory tract, the natural site of colonization by this bacterium, is imperative. We have previously observed that immunity conferred by prior infection is superior to parenteral vaccination in preventing colonization by *B. bronchiseptica* in a mouse model (12). Infections, but not parenteral vaccines, induce a strong IgA response (12), suggesting that mucosal IgA may render protection conferred by infection-induced immunity more efficiently than that conferred by parenteral vaccines. In order to address this possibility, wild-type and IgA$^{-/-}$ mice were inoculated with *B. bronchiseptica* as above. Beginning on day 21 postinoculation, mice were treated with gentamicin for 7 days. This treatment completely eliminated bacteria from the lungs and trachea, and only 10 to 100 bacteria remained in the nasal cavities on day 28 postinoculation. These mice were challenged with gentamicin-resistant (GENT^r) *B. bronchiseptica* on day 30 postinoculation. On day 3 postchallenge, the numbers of *B. bronchiseptica* and GENT^r *B. bronchiseptica* recovered from various respiratory organs were measured. Bacterial numbers from the secondary infection (GENT^r *B. bronchiseptica*) were reduced to levels near the lower limit of detection in the lungs of wild-type and IgA$^{-/-}$ mice (Fig. 3). While numbers were also near the lower limit of detection in the tracheae of wild-type mice, the tracheae of IgA$^{-/-}$ mice harbored ~10^4 CFU of GENT^r *B. bronchiseptica* (Fig. 3). Similarly, wild-type mice harbored only hundreds of streptomycin-resistant (STR^r) *B. bronchiseptica* and GENT^r *B. bronchiseptica* in the nasal cavities, whereas IgA$^{-/-}$ mice harbored ~10^3 CFU of STR^r *B. bronchiseptica* (data not shown) and ~10^6 CFU of GENT^r *B. bronchiseptica* in their nasal cavities (Fig. 3). Similar results were observed on day 7 postchallenge (data not shown). These data indicate that the immune response induced by infection of IgA$^{-/-}$ mice failed to prevent subsequent *B. bronchiseptica* colonization in the tracheae and nasal cavities.

As individuals vaccinated against whooping cough are able to be infected by *B. pertussis* and *B. parapertussis* (6), we sought to test whether IgA is important to the prevention of subsequent infections by these pathogens. Mice were inoculated

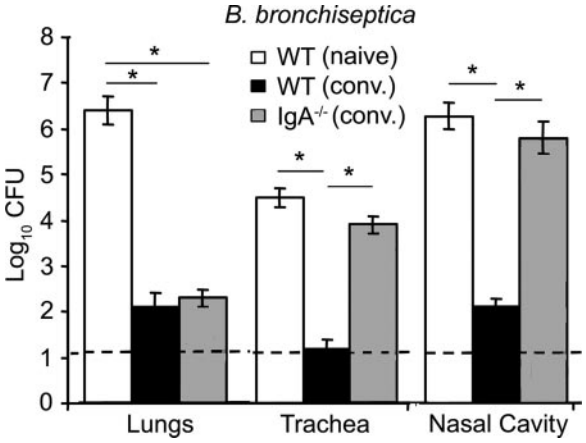

FIG. 3. Colonization of convalescent wild type (WT) and $IgA^{-/-}$ mice by *B. bronchiseptica*. Groups of four C57BL/6 (WT conv.) and $IgA^{-/-}$ ($IgA^{-/-}$ conv.) mice were inoculated with 5×10^5 CFU STR^r *B. bronchiseptica*. These mice were treated with gentamicin for 7 days beginning on day 21 postinoculation. On day 30, mice were challenged with 5×10^5 CFU of $GENT^r$ *B. bronchiseptica*. Mice were sacrificed on day 3 postchallenge, and respiratory organs were harvested. The numbers of $GENT^r$ *B. bronchiseptica* were quantified in the lungs, tracheae, and nasal cavities and compared to *B. bronchiseptica* numbers in naïve C57BL/6 mice (WT naïve). Bacterial numbers are expressed as the mean $\log_{10}$ CFU ± the standard error (error bars). The dashed line represents the lower limit of detection.

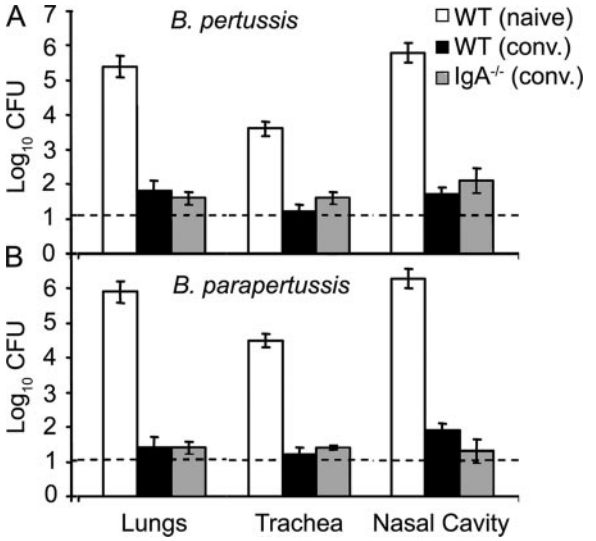

FIG. 4. Colonization of convalescent wild-type (WT) and $IgA^{-/-}$ mice by *B. pertussis* and *B. parapertussis*. Groups of four C57BL/6 (WT conv.) and $IgA^{-/-}$ ($IgA^{-/-}$ conv.) mice were inoculated with 5×10^5 CFU STR^r *B. pertussis* or STR^r *B. parapertussis*. These mice were treated with gentamicin for 7 days beginning on day 21 postinoculation. On day 30, mice were challenged with 5×10^5 CFU of $GENT^r$ *B. pertussis* (A) or $GENT^r$ *B. parapertussis* (B), respectively. Mice were sacrificed on day 3 postchallenge, and respiratory organs were harvested. The numbers of gentamicin-resistant bacteria were quantified in the lungs, tracheae, and nasal cavities and compared to those in naïve C57BL/6 mice (WT naïve). The numbers of gentamicin-resistant bacteria are expressed as the mean $\log_{10}$ CFU ± the standard error (error bars). The dashed line represents the lower limit of detection.

with *B. pertussis* or *B. parapertussis*, then treated with gentamicin as above and, on day 30, challenged with $GENT^r$ *B. pertussis* or $GENT^r$ *B. parapertussis*. In contrast to the results observed with *B. bronchiseptica*, $GENT^r$ *B. pertussis* numbers were reduced to less than 100 CFU in the lungs, tracheae, and nasal cavities of wild-type and $IgA^{-/-}$ convalescent mice alike (Fig. 4A). Similarly, $GENT^r$ *B. parapertussis* numbers were reduced to less than 100 CFU in the lungs, tracheae, and nasal cavities of wild-type and $IgA^{-/-}$ convalescent mice (Fig. 4B). IgA is induced upon infection by the human-adapted bordetellae (data not shown) (42) but does not appear to be important to preventing subsequent infections by these pathogens in the mouse model.

$IgA^{-/-}$ mice show normal serum antibody responses to *B. bronchiseptica*. $IgA^{-/-}$ mice have an altered antibody isotype profile, producing elevated IgG2b and decreased IgG3 in response to influenza viral infection (13). Thus, one possible explanation for the inability of $IgA^{-/-}$ mice to clear *B. bronchiseptica* infections from the trachea and nasal cavity could be that these mice have an altered serum antibody response to this pathogen. Therefore, we compared the titers of *B. bronchiseptica*-specific antibodies and isotypes in the sera of wild-type and $IgA^{-/-}$ mice (collected day 28 postinoculation) inoculated with *B. bronchiseptica*. The overall titers of serum antibodies in wild-type mice were not significantly different from those observed in $IgA^{-/-}$ mice (Fig. 5). Similarly, titers of anti-*Bordetella* IgM, IgG, IgG1, IgG2a, IgG2b, and IgG3 were indistinguishable between wild-type and $IgA^{-/-}$ mice (Fig. 5). These results suggest that the defect in controlling bacterial numbers in the tracheae and nasal cavities of $IgA^{-/-}$ mice was not due to altered production of other antibody isotypes and support the hypothesis that mucosal IgA is essential to reducing the numbers of *B. bronchiseptica* in the upper respiratory tract.

Serum antibody-mediated clearance of *B. bronchiseptica* from the trachea requires IgA. Previously we showed that passively transferred serum antibodies rapidly clear *B. bronchiseptica* from the lungs and tracheae of mice (20). Here we sought to determine the contribution of serum IgA to the

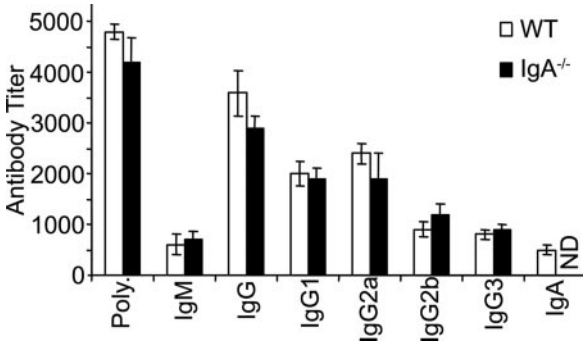

FIG. 5. *B. bronchiseptica*-specific antibody titers in sera of wild-type (WT) and $IgA^{-/-}$ mice inoculated with *B. bronchiseptica*. Groups of C57BL/6 and $IgA^{-/-}$ mice were inoculated with 5×10^5 CFU of *B. bronchiseptica*. On day 28 postinoculation, sera were collected from these mice and titers of anti-*B. bronchiseptica* antibodies (Poly) were determined by ELISA along with titers of specific isotypes. Antibody titers are expressed as the mean endpoint titer ± standard error (error bars).

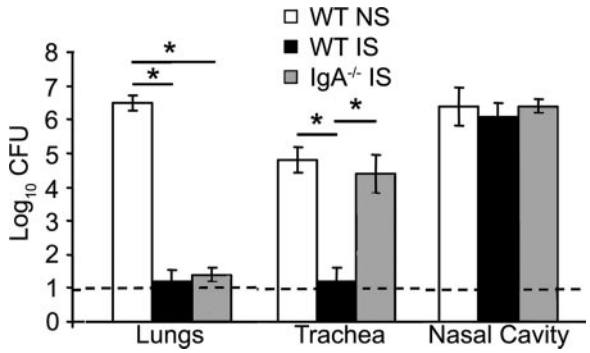

FIG. 6. Effect of convalescent-phase serum from wild-type (WT) versus $IgA^{-/-}$ mice on colonization by *B. bronchiseptica*. Groups of four C57BL/6 mice were inoculated with 5×10^5 CFU of *B. bronchiseptica* and immediately given passive transfers of 200 μl of naïve serum (WT NS) or wild-type (WT IS) or $IgA^{-/-}$ ($IgA^{-/-}$ IS) convalescent-phase serum intraperitoneally. On day 3 postinoculation, respiratory organs were harvested and bacterial numbers were quantified in the lungs, tracheae, and nasal cavities. Bacterial numbers are expressed as the mean $\log_{10}$ CFU ± the standard error (error bars). The dashed line represents the lower limit of detection.

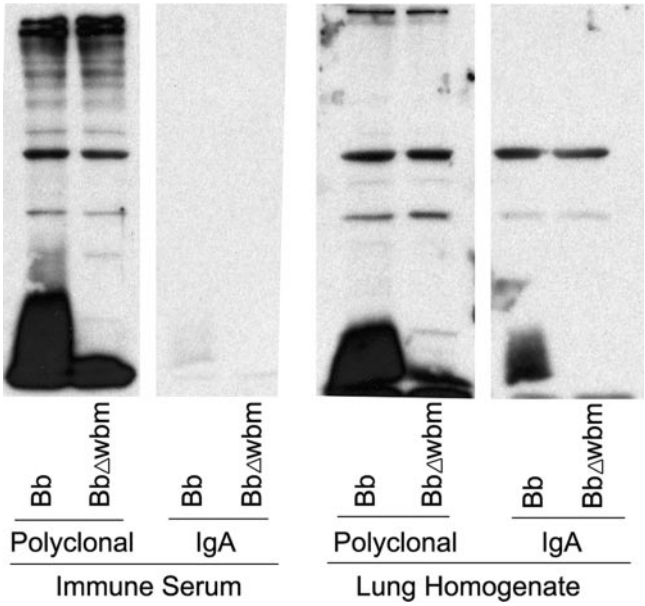

FIG. 7. Recognition of *B. bronchiseptica* antigens by antibodies in convalescent-phase serum or lung homogenate. Groups of three C57BL/6 mice were inoculated with 5×10^5 CFU of *B. bronchiseptica*. Twenty-eight days later, sera and lungs were collected. Western blot assays were performed using lysates from wild-type *B. bronchiseptica* (Bb) or the O-antigen-deficient strain (BbΔwbm) probed with pooled convalescent-phase serum or lung homogenate. Horseradish peroxidase-conjugated secondary antibodies specific for all murine antibody isotypes (polyclonal) or only IgA were used for detection.

antibody-mediated clearance of *B. bronchiseptica*. Groups of four wild-type mice were inoculated with *B. bronchiseptica* and given a passive transfer of 200 μl of naïve or convalescent-phase serum from wild-type or $IgA^{-/-}$ mice. On day 3 postinoculation, naïve serum-treated mice harbored approximately 10^6 and 10^4 CFU of *B. bronchiseptica* in the lungs and trachea, respectively. Convalescent-phase serum from wild-type mice reduced *B. bronchiseptica* numbers in the lungs and trachea nearly to the limit of detection by day 3 postinoculation (Fig. 6). IgA-deficient serum reduced *B. bronchiseptica* to less than 100 CFU in the lungs, but approximately 10^4 bacteria were still present in the trachea (Fig. 6). As previously observed, passively transferred antibodies had no effect in the nasal cavities of mice (20). Together, these data indicate that IgA is important to serum antibody-mediated clearance of *B. bronchiseptica* from the trachea.

The IgA response is primarily directed against the O-antigen of *B. bronchiseptica*. Since IgA was found to be necessary for efficient reduction of bacterial numbers in the upper respiratory tract, we sought to determine the bacterial factors that were being recognized by IgA. Western blots with lysates of wild-type *B. bronchiseptica* or an isogenic mutant lacking O-antigen were probed with serum or lung homogenate from convalescent wild-type mice. Horseradish peroxidase-conjugated antibodies specific for all murine antibody isotypes (polyclonal) or IgA only were used to identify the antigens that were bound by IgA. We found that IgA in the serum and lungs primarily recognized a low-molecular-weight smear that was present in the wild-type *B. bronchiseptica* strain but absent from the strain lacking O-antigen (Fig. 7). This indicated that IgA induced by *B. bronchiseptica* infection primarily recognizes the O-antigen of that bacterium.

O-antigen-specific antibodies are important to clearance of *B. bronchiseptica* from the trachea. The fact that IgA was protective in the trachea and recognized O-antigen led us to speculate that antibodies against O-antigen are important to bacterial clearance from the trachea. Serum raised against the O-antigen-deficient *B. bronchiseptica* strain did not contain antibodies to all antigens that were recognized by serum from mice infected with the wild-type strain but did contain antibodies to antigens that are known to be protective antigens of *B. bronchiseptica* (data not shown) (24). The largest difference between the sera was the absence of O-antigen-specific antibodies in the serum raised against the O-antigen-deficient strain. To test if O-antigen is an important protective antigen, groups of four wild-type mice were inoculated with *B. bronchiseptica* and given a passive transfer of serum raised against wild-type *B. bronchiseptica* or an isogenic *B. bronchiseptica* strain lacking O-antigen. Mice were then sacrificed 3 days later to quantify bacterial numbers throughout the respiratory tract. Mice given passive transfers of serum raised against wild-type or O-antigen-deficient *B. bronchiseptica* were able to reduce bacterial numbers in the lungs to less than 100 CFU within 3 days. Serum raised against wild-type bacteria reduced *B. bronchiseptica* numbers in the trachea, but serum raised against the O-antigen-deficient strain was ineffective against *B. bronchiseptica* in the trachea (Fig. 8). Along with the facts that serum IgA is required for clearance of *B. bronchiseptica* from the trachea and primarily recognizes O-antigen, these data suggest that O-antigen-specific IgA mediates bacterial clearance from the trachea. Interestingly, serum raised against the O-antigen-deficient strain appeared to have a greater effect on bacterial numbers in the nasal cavity than serum raised against wild-type bacteria (Fig. 8).

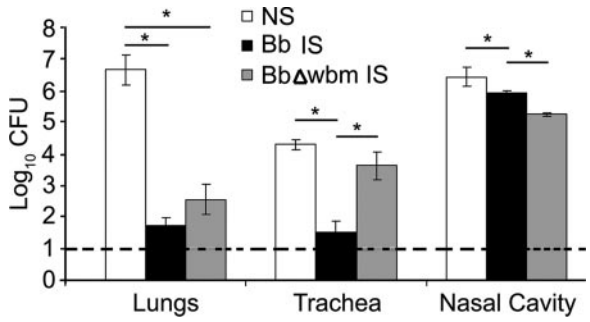

FIG. 8. Effects of antibodies against O-antigen on colonization by *B. bronchiseptica*. Groups of four C57BL/6 mice were inoculated with 5×10^5 CFU of *B. bronchiseptica* and immediately given passive transfers of 200 μl of naïve serum (NS), *B. bronchiseptica* convalescent-phase serum (Bb IS), or *B. bronchiseptica*Δ*wbm* convalescent-phase serum (BbΔwbm IS). On day 3 postinoculation, respiratory organs were harvested and bacterial numbers were quantified in the lungs, tracheae, and nasal cavities. Bacterial numbers are expressed as the mean $\log_{10}$ CFU ± the standard error (error bars). The dashed line represents the lower limit of detection.

DISCUSSION

Although we have a relatively detailed understanding of the mechanisms by which *B. bronchiseptica*, *B. pertussis*, and *B. parapertussis* are eliminated from the lower respiratory tract (20, 21, 42), less is known about how these pathogens are controlled in the nasal cavity. Here we show that IgA plays an important role in controlling *B. bronchiseptica* numbers in the upper respiratory tract. IgA induced upon infection by *B. bronchiseptica* recognized a limited number of antigens and was largely specific for the O-antigen of this bacterium, and both IgA and O-antigen-specific antibodies were important to eliminating *B. bronchiseptica* from the trachea. In contrast, IgA did not appear to have a protective role against *B. pertussis* or *B. parapertussis*. While IgA may not be important to protecting against the human-adapted bordetellae, it is also possible that this is an artifact of the mouse model, as we will discuss below.

IgA can mediate protection against bacterial and viral pathogens through a number of mechanisms. Secretory IgA is a part of the first line of defense, neutralizing and agglutinating pathogens at the mucosal surface (30, 38). Serum IgA may act as a second line of defense, acting against pathogens that have breached the mucosal surface (30, 38). Both forms of IgA modulate local inflammation, although secretory IgA appears to induce anti-inflammatory signals and serum IgA induces proinflammatory signals (3, 38). Serum IgA also facilitates antibody-dependent cell-mediated cytotoxicity and Fcα-mediated phagocytosis (29, 40). Our data suggest that serum IgA, although there is little IgA in the serum of *B. bronchiseptica*-convalescent mice, is necessary for protection against *B. bronchiseptica* in the trachea, since a passive transfer of serum antibodies from wild-type mice led to a reduction of bacterial numbers but serum from $IgA^{-/-}$ mice did not (Fig. 6). The fact that a similar trend was not observed in the nasal cavity upon the passive transfer of antibodies could have been the result of inefficient transport of serum antibodies to the nasal epithelium. Alternatively, secretory IgA may be more important to protection in the nasal cavity while serum IgA may mediate bacterial clearance from the trachea. That being said, the specific roles of secretory and serum IgA upon *B. bronchiseptica* remain unclear.

Mucosal bacterial pathogens, such as *Streptococcus*, *Neisseria*, and *Bordetella* species, continue to circulate despite the widespread use of vaccines (5, 9, 14, 28, 37, 41). This may, in part, be attributed to current vaccine strategies which are aimed at inducing serum antibodies to specific virulence factors and therefore protect against severe disease but do not prevent infection. We and others have observed that serum antibodies are ineffective against some mucosal pathogens in the upper respiratory tract (20, 34). It is therefore important for vaccines to elicit appropriate local immune responses that can prevent colonization by pathogens if there is a desire to not only protect from disease but also prevent infection. Because of this, some recent immunization strategies have emphasized the induction of a strong, local IgA response using mucosal vaccines to try to curb the spread of infectious diseases.

Care must be taken when extrapolating studies in a mouse model to human models of infection and disease. There are substantial differences in IgA-mediated immunity between mice and humans regarding the IgA receptors that they express (8). Although IgA was not required to clear *B. pertussis* and *B. parapertussis* in the mouse model, this isotype may be important in humans. For example, *B. parapertussis* appears to persist for life in the nasal cavity in the murine model (20, 42), but this does not appear to be the case in humans. Thus, clearance of *B. parapertussis* from the human nasal cavity may be dependent on some IgA-dependent mechanism that is lacking in mice. Additionally, Hellwig et al. showed that human Fcα receptor I-transgenic mice are able to more efficiently control *B. pertussis* than nontransgenic mice (16), suggesting that IgA-mediated immunity to *B. pertussis* may be more important in human hosts. Thus, whooping cough vaccines that induce *Bordetella*-specific IgA could prove to be more efficacious than current strategies (10).

Unlike the human-adapted bordetellae, *B. bronchiseptica* naturally colonizes and chronically persists in the nasal cavity and trachea of a variety of mammals (11), and IgA mediates protection in these respiratory compartments. Poor induction of *B. bronchiseptica*-specific IgA could partially explain the lack of efficacy of current *B. bronchiseptica* vaccines. Some current vaccine strategies involve the use of live, attenuated *B. bronchiseptica* strains (26), and we have recently shown that infection by a genetically defined avirulent *B. bronchiseptica* strain, AVS, confers efficient immunity to *B. bronchiseptica* (24). Utilizing vaccine strategies consisting of intranasal inoculations with live, attenuated strains that induce strong, *B. bronchiseptica*-specific IgA responses may aid in preventing the spread of this pathogen among domestic animal populations.

ACKNOWLEDGMENTS

This study was supported by NIH grant AI 053075 (E.T.H.).

We have no conflicting financial interests.

We thank Innocent Mbawuike for the generous donation of $IgA^{-/-}$ mice.

REFERENCES

1. **Akhiani, A. A., A. Stensson, K. Schon, and N. Y. Lycke.** 2005. IgA antibodies impair resistance against *Helicobacter pylori* infection: studies on immune evasion in IL-10-deficient mice. J. Immunol. **174:**8144–8153.
2. **Allen, A. G., R. M. Thomas, J. T. Cadisch, and D. J. Maskell.** 1998. Molec-

ular and functional analysis of the lipopolysaccharide biosynthesis locus wlb from *Bordetella pertussis*, *Bordetella parapertussis* and *Bordetella bronchiseptica*. Mol. Microbiol. **29:**27–38.
3. **Arnaboldi, P. M., M. J. Behr, and D. W. Metzger.** 2005. Mucosal B cell deficiency in $IgA^{-/-}$ mice abrogates the development of allergic lung inflammation. J. Immunol. **175:**1276–1285.
4. **Bemis, D. A., and J. R. Kennedy.** 1981. An improved system for studying the effect of *Bordetella bronchiseptica* on the ciliary activity of canine tracheal epithelial cells. J. Infect. Dis. **144:**349–357.
5. **Celentano, L. P., M. Massari, D. Paramatti, S. Salmaso, A. E. Tozzi, et al.** 2005. Resurgence of pertussis in Europe. Pediatr. Infect. Dis. J. **24:**761–765.
6. **Cherry, J. D., D. X. Xing, P. Newland, K. Patel, U. Heininger, and M. J. Corbel.** 2004. Determination of serum antibody to *Bordetella pertussis* adenylate cyclase toxin in vaccinated and unvaccinated children and in children and adults with pertussis. Clin. Infect. Dis. **38:**502–507.
7. **Cotter, P. A., and J. Miller.** 1994. BvgAS-mediated signal transduction: analysis of phase-locked regulatory mutants of *Bordetella bronchiseptica* in a rabbit model. Infect. Immun. **62:**3381–3390.
8. **Decot, V., G. Woerly, M. Loyens, S. Loiseau, B. Quatannens, M. Capron, and D. Dombrowicz.** 2005. Heterogeneity of expression of IgA receptors by human, mouse, and rat eosinophils. J. Immunol. **174:**628–635.
9. **de Melker, H. E., J. F. Schellekens, S. E. Neppelenbroek, F. R. Mooi, H. C. Rumke, and M. A. Conyn-van Spaendonck.** 2000. Reemergence of pertussis in the highly vaccinated population of the Netherlands: observations on surveillance data. Emerg. Infect. Dis. **6:**348–357.
10. **Elahi, S., R. M. Buchanon, L. A. Babiuk, and V. Gerdts.** 2006. Maternal immunity provides protection against pertussis in newborn piglets. Infect. Immun. **74:**2619–2627.
11. **Goodnow, R. A.** 1980. Biology of *Bordetella bronchiseptica*. Microbiol. Rev. **44:**722–738.
12. **Gopinathan, L., G. S. Kirimanjeswara, D. N. Wolfe, M. L. Kelley, and E. T. Harvill.** 2007. Different mechanisms of vaccine-induced and infection-induced immunity to *Bordetella bronchiseptica*. Microbes Infect. **9:**442–448.
13. **Harriman, G. R., M. Bogue, P. Rogers, M. Finegold, S. Pacheco, A. Bradley, Y. Zhang, and I. N. Mbawuike.** 1999. Targeted deletion of the IgA constant region in mice leads to IgA deficiency with alterations in expression of other Ig isotypes. J. Immunol. **162:**2521–2529.
14. **Harrison, L. H., K. A. Jolley, K. A. Shutt, J. W. Marsh, M. O'Leary, L. T. Sanza, M. C. Maiden, et al.** 2006. Antigenic shift and increased incidence of meningococcal disease. J. Infect. Dis. **193:**1266–1274.
15. **Heininger, U., P. A. Cotter, H. W. Fescemyer, G. Martinez de Tejada, M. H. Yuk, J. F. Miller, and E. T. Harvill.** 2002. Comparative phenotypic analysis of the *Bordetella parapertussis* isolate chosen for genomic sequencing. Infect. Immun. **70:**3777–3784.
16. **Hellwig, S. M., A. B. van Spriel, J. F. Schellekens, F. R. Mooi, and J. G. van de Winkel.** 2001. Immunoglobulin A-mediated protection against *Bordetella pertussis* infection. Infect. Immun. **69:**4846–4850.
17. **Horton, R. E., J. Stuart, H. Christensen, R. Borrow, T. Guthrie, V. Davenport, A. Finn, N. A. Williams, R. S. Heyderman, et al.** 2005. Influence of age and carriage status on salivary IgA to *Neisseria meningitidis*. Epidemiol. Infect. **133:**883–889.
18. **Iankov, I. D., D. P. Petrov, I. V. Mladenov, I. H. Haralambieva, O. K. Kalev, M. S. Balabanova, and I. G. Mitov.** 2004. Protective efficacy of IgA monoclonal antibodies to O and H antigens in a mouse model of intranasal challenge with *Salmonella enterica* serotype Enteritidis. Microbes Infect. **6:**901–910.
19. **Janoff, E. N., C. Fasching, J. M. Orenstein, J. B. Rubins, N. L. Opstad, and A. P. Dalmasso.** 1999. Killing of *Streptococcus pneumoniae* by capsular polysaccharide-specific polymeric IgA, complement, and phagocytes. J. Clin. Investig. **104:**1139–1147.
20. **Kirimanjeswara, G. S., P. Mann, and E. T. Harvill.** 2003. Role of antibodies in immunity to *Bordetella* infections. Infect. Immun. **71:**1719–1724.
21. **Kirimanjeswara, G. S., P. Mann, M. Pilione, M. J. Kennett, and E. T. Harvill.** 2005. The complex mechanism of antibody-mediated clearance of *Bordetella* from the lungs requires TLR4. J. Immunol. **175:**7504–7511.
22. **Maaser, C., M. P. Housley, M. Iimura, J. R. Smith, B. A. Vallance, B. B. Finlay, J. R. Schreiber, N. M. Varki, M. F. Kagnoff, and L. Eckmann.** 2004. Clearance of *Citrobacter rodentium* requires B cells but not secretory immunoglobulin A (IgA) or IgM antibodies. Infect. Immun. **72:**3315–3324.
23. **Magyar, T., N. Chanter, A. J. Lax, J. M. Rutter, and G. A. Hall.** 1988. The pathogenesis of turbinate atrophy in pigs caused by *Bordetella bronchiseptica*. Vet. Microbiol. **18:**135–146.
24. **Mann, P. B., E. M. Goebel, J. Barbarich, M. Pilione, M. Kennett, and E. T. Harvill.** 2007. Use of a genetically defined double mutant strain of *B. bronchiseptica* lacking adenylate cyclase and type III secretion as a live vaccine. Infect. Immun. **75:**3665–3672.
25. **Mastrantonio, P., P. Stefanelli, M. Giuliano, Y. Herrera Rojas, M. Ciofi degli Atti, A. Anemona, and A. E. Tozzi.** 1998. *Bordetella parapertussis* infection in children: epidemiology, clinical symptoms, and molecular characteristics of isolates. J. Clin. Microbiol. **36:**999–1002.
26. **Mattoo, S., and J. Cherry.** 2005. Molecular pathogenesis, epidemiology, and clinical manifestations of respiratory infections due to *Bordetella pertussis* and other *Bordetella* subspecies. Clin. Microbiol. Rev. **18:**326–382.
27. **Mbawuike, I. N., S. Pacheco, C. L. Acuna, K. C. Switzer, Y. Zhang, and G. R. Harriman.** 1999. Mucosal immunity to influenza without IgA: an IgA knockout mouse model. J. Immunol. **162:**2530–2537.
28. **Melegaro, A., W. J. Edmunds, R. Pebody, E. Miller, and R. George.** 2006. The current burden of pneumococcal disease in England and Wales. J. Infect. **52:**37–48.
29. **Monteiro, R. C., and J. G. van de Winkel.** 2003. IgA Fc receptors. Annu. Rev. Immunol. **21:**177–204.
30. **Otten, M. A., and M. van Egmond.** 2004. The Fc receptor for IgA (FcαRI, CD89). Immunol. Lett. **92:**23–31.
31. **Pennington, J. E., G. J. Small, M. E. Lostrom, and G. B. Pier.** 1986. Polyclonal and monoclonal antibody therapy for experimental *Pseudomonas aeruginosa* pneumonia. Infect. Immun. **54:**239–244.
32. **Preston, A., A. G. Allen, J. Cadisch, R. Thomas, K. Stevens, C. M. Churcher, K. L. Badcock, J. Parkhill, B. Barrell, and D. J. Maskell.** 1999. Genetic basis for lipopolysaccharide O-antigen biosynthesis in bordetellae. Infect. Immun. **67:**3763–3767.
33. **Relman, D., E. Tuomanen, S. Falkow, D. T. Golenbock, K. Saukkonen, and S. D. Wright.** 1990. Recognition of a bacterial adhesion by an integrin: macrophage CR3 (alpha M beta 2, CD11b/CD18) binds filamentous hemagglutinin of *Bordetella pertussis*. Cell **61:**1375–1382.
34. **Renegar, K. B., P. A. Small, L. G. Boykins, and P. F. Wright.** 2004. Role of IgA versus IgG in the control of influenza viral infection in the murine respiratory tract. J. Immunol. **173:**1978–1986.
35. **Robbins, J. B., C. Chu, and R. Schneerson.** 1992. Hypothesis for vaccine development: protective immunity to enteric diseases caused by nontyphoidal salmonellae and shigellae may be conferred by serum IgG antibodies to the O-specific polysaccharide of their lipopolysaccharides. Clin. Infect. Dis. **15:**346–361.
36. **Rodriguez, A., A. Tjarnlund, J. Ivanji, M. Singh, I. Garcia, A. Williams, P. D. Marsh, M. Troye-Blomberg, and C. Fernandez.** 2005. Role of IgA in the defense against respiratory infections IgA deficient mice exhibited increased susceptibility to intranasal infection with *Mycobacterium bovis* BCG. Vaccine **23:**2565–2572.
37. **Skowronski, D. M., G. De Serres, D. MacDonald, W. Wu, C. Shaw, J. Macnabb, S. Champagne, D. M. Patrick, and S. A. Halperin.** 2002. The changing age and seasonal profile of pertussis in Canada. J. Infect. Dis. **185:**1448–1453.
38. **Snoeck, V., I. R. Peters, and E. Cox.** 2006. The IgA system: a comparison of structure and function in different species. Vet. Res. **37:**455–467.
39. **Sun, K., F. E. Johansen, L. Eckmann, and D. W. Metzger.** 2004. An important role for polymeric Ig receptor-mediated transport of IgA in protection against *Streptococcus pneumoniae* nasopharyngeal carriage. J. Immunol. **173:**4576–4581.
40. **van Egmond, M., C. A. Damen, A. B. van Spriel, G. Vidarsson, E. van Garderen, and J. G. van de Winkel.** 2001. IgA and the IgA Fc receptor. Trends Immunol. **22:**205–211.
41. **von Konig, C. H., S. Halperin, M. Riffelmann, and N. Guiso.** 2002. Pertussis of adults and infants. Lancet Infect. Dis. **2:**744–750.
42. **Wolfe, D. N., G. Kirimanjeswara, and E. T. Harvill.** 2005. Clearance of *Bordetella parapertussis* from the lower respiratory tract requires humoral and cellular immunity. Infect. Immun. **73:**6508–6513.

Editor: D. L. Burns