Immunoglobulin A-Mediated Protection against 
Bordetella pertussis Infection

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Infection with Bordetella pertussis, the causative agent of pertussis (whooping cough), in humans, is followed by the production of antibodies of several isotypes, including immunoglobulin A (IgA). Little is known, however, about the role of IgA in immunity against pertussis. Therefore, we studied targeting of B. pertussis to the myeloid receptor for IgA, FcεRI (CD89), using either IgA purified from immune sera of pertussis patients or bispecific antibodies directed against B. pertussis and FcεRI (CD89 BsAb). Both IgA and CD89 BsAb facilitated FcεRI-mediated binding, phagocytosis, and bacterial killing by human polymorphonuclear leukocytes (PMNL) and PMNL originating from human FcεRI-transgenic mice. Importantly, FcεRI targeting resulted in enhanced bacterial clearance in lungs of transgenic mice. These data support the capacity of IgA to induce anti-B. pertussis effector functions via the myeloid IgA receptor, FcεRI. Increasing the amount of IgA antibodies induced by pertussis vaccines may result in higher vaccine efficacy.

The gram-negative bacterium Bordetella pertussis is the causative agent of pertussis (whooping cough). B. pertussis expresses various virulence factors, including adhesins and toxins, which all play a role in pathogenesis. B. pertussis colonizes the respiratory tract using adhesins specific for ciliated cells of the respiratory epithelium. Toxins are produced and are involved in disrupting host immune responses (21). The mechanisms underlying immunity to B. pertussis are incompletely understood. In murine infection models, protection against infection was obtained upon passive transfer of anti-B. pertussis antibodies (8, 10, 23). In addition, protective effects of T helper 1 cells (2, 16) and B cells (14) have been observed, indicating that antibodies, B cells, and T cells are involved in protective immunity.

Protection against bacterial infections depends on effector activities by phagocytic cells. Elimination of bacteria involves opsonization with antibodies and recognition by certain receptors that may result in phagocytosis, bacterial killing, and antigen presentation. Upon B. pertussis infection in humans, antibody levels rise, and high levels in acute-phase sera have been associated with a lower likelihood of acquiring pertussis (3, 5, 24). Anti-B. pertussis antibodies consist of different isotypes, including immunoglobulin A (IgA) (19, 37). B. pertussis is non-invasive and is found exclusively on mucosa of the respiratory tract. Since IgA represents the predominant antibody isotype at mucosal surfaces, a role for IgA in anti-B. pertussis mechanisms is possible.

IgA is generally believed to function by neutralizing and agglutinating pathogens or by preventing their attachment to mucosal surfaces (4, 12). The role of IgA, however, may be much broader because of effector functions induced by binding to IgA receptors. The prototypic IgA receptor (FcεRI [CD89]) is found exclusively on cells of the myeloid lineage: monocytes, macrophages, neutrophils, and eosinophils (13, 15, 17). Increasing evidence shows that FcεRI exhibits potent proinflammatory capacities. FcεRI cross-linking readily induces phagocytosis, degranulation, respiratory burst, antibody-dependent cellular cytotoxicity, and the release of proinflammatory cytokines (31).

The aim of the present study was to evaluate IgA-mediated effector functions against B. pertussis by studying the interaction of IgA-coated B. pertussis with human polymorphonuclear leukocytes (PMNL). In addition, experiments were performed with transgenic (Tg) mice expressing the human FcεRI (28). There is no known homologue of FcεRI in mice, and CD89-Tg mice have been used to study the in vivo role of human FcεRI (29). We demonstrate that anti-B. pertussis IgA exhibits bactericidal effector function via facilitation of binding, phagocytosis, and killing of B. pertussis involving FcεRI.

MATERIALS AND METHODS

Mice. FcεRI (CD89) transgenic mice, were crossed with C57BL/6 mice, and experiments were performed with F1 generation Tg mice and nontransgenic (NTg) littermates. Similar to the situation in humans, FcεRI in these mice is constitutively expressed on PMNL and is inducible on macrophages (28). Both male and female mice were used at between 5 and 9 weeks of age. Mice were maintained under supervision of the institutes council for experiments on animals (DEC), according to Dutch legislation.

Bacterial strains and growth conditions. B. pertussis strain B213 was used for the experiments and is a streptomycin-resistant derivative of strain Tohama. Bacteria were stored at −70°C, recovered by growth on Bordet Gengou (BG) agar plates supplemented with 30 μg of streptomycin per ml at 35°C for 3 days, and used for in vitro experiments. For infection of mice, strains were subsequently plated on BG plates without antibiotics, cultured for 3 days, and used for infection.

Antibodies. Sera of pertussis patients with high B. pertussis-specific IgA titers (measured by IgA enzyme-linked immunosorbent assay as described in reference
19) were pooled. IgA antibodies were subsequently purified using Affi-T columns (Biozym, Landgraaf, The Netherlands) and separated by size chromatography (Superdex 200, Pharmacia, Piscataway, N.J.). Fractions were analyzed by electrophoresis on sodium dodecyl sulfate–4 to 15% gradient gels (Phast gel; Pharmacia) and Coomassie brilliant blue staining. To exclude the presence of other isotypes, Western blot analyses were performed. Anti-FcRII (A77; murine IgG1) was obtained from Medarex (Annandale, N.J.). FcRII-blocking monoclonal antibody (2D11; murine IgG1) was a generous gift of G. van Zandbergen (18). Rabbits were immunized with pertussis whole-cell vaccine (RIVM, Bilthoven, The Netherlands) to generate polyclonal rabbit anti-

B. pertussis antibodies were removed by washing, and bacteria were incubated with PMNL and erythrocytes were removed by hypotonic lysis. Cells were washed twice with RPMI 1640 medium supplemented with 10% fetal calf serum, counted, and used immediately.

Mouse PMNL were obtained as described previously (30). In short, mice were lightly anesthetized with ether, and 40 mL of inoculum (containing 10B. pertussis organisms) was inhaled. Prior to infection, the numbers of CFU in the inocula were determined by plating on BG plates. To assess bacterial colonization, groups of mice were killed by intramuscular injection of an overdose of pentobarbital sodium (Nembutal; Sanofi, Maasland, The Netherlands) 2 days after infection. Lungs were excised and then homogenized using a blender in 900 mL of Verwey medium. Viable bacteria in homogenized organs were determined by plating serial dilutions on BG agar plates supplemented with 30 g/mL of streptomycin per mL.

Statistical analyses. Means and standard deviations were calculated from log_{10}-transformed numbers of CFU. Differences between various groups were assessed by two-tailed Student t tests with significance at a p value of <0.05.

RESULTS

IgA enhances PMNL phagocytosis of B. pertussis. To study IgA-mediated cellular effector functions, we evaluated whether IgA antibodies facilitated binding and uptake of B. pertussis by PMNL. First, human anti-B. pertussis serum IgA was purified and its opsonic potential was investigated. B. pertussis cells were incubated with human IgA, and binding was visualized with FITC-conjugated secondary antibodies (a-chain specific). Purified anti-B. pertussis IgA bound B. pertussis efficiently as detected by flow cytometry (Fig. 1A). We next generated BsAb, consisting of one arm directed to B. pertussis and one directed to FcRII (CD89) for B. pertussis (18). Rabbits were immunized with pertussis whole-cell vaccine (RIVM, Bilthoven, The Netherlands) to generate polyclonal rabbit anti-

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Phagocytosis of FcRII-directed B. pertussis was analyzed using flow cytometry. B. pertussis was labeled with PKH-26, a red fluorescent marker detectable in the phagocytin channel, opsonized with human anti-B. pertussis IgA antibodies, and incubated with PMNL. In our flow cytometric assay, PKH-26 fluorescence reflects PMNL binding and phagocytosis of B. pertussis, whereas FITC fluorescence selectively assays nonopsonized (surface-bound) bacteria. Phagocytosis of IgA-opsonized B. pertussis was assessed using PMNL from human FcRII-Tg mice and PMNL from NTg littermates (controls). IgA enhanced binding and subsequent phagocytosis of

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B. pertussis by Tg PMNL, as was reflected by decreased FITC fluorescence after a temperature shift from 4 to 37°C (Fig. 2A and B). In selected experiments, cytochalasin D was added during the assay to inhibit internalization. Phagocytosis was subsequently inhibited, which was indicated by residual high FITC fluorescence after incubation at 37°C (not shown). Phagocytosis was largely mediated by FcαRI, since NTg (control) PMNL bound IgA-opsonized B. pertussis less efficiently and attached bacteria were phagocytosed less well (Fig. 2B). Similar experiments were performed with human PMNL. Serum IgA promoted uptake and phagocytosis of B. pertussis by human PMNL, which was blocked by FcαRI-blocking antibody 2D11 (Fig. 2C). To prove phagocytosis to be truly triggered by IgA-FcαRI interactions, similar experiments were performed with CD89 BsAb-opsonized B. pertussis, yielding identical results (data not shown).

**Bactericidal activity mediated via FcαRI.** We next investigated whether phagocytosis via FcαRI induces PMNL-mediated bacterial killing. Tg and NTg PMNL were allowed to internalize nonopsonized or CD89 BsAb-opsonized B. pertussis, and numbers of killed bacteria were determined. Both Tg and NTg PMNL killed B. pertussis, but FcαRI targeting significantly increased the numbers of killed bacteria (Fig. 3).

Anti-B. pertussis activity via FcαRI was also analyzed in vivo by infecting both Tg and NTg mice with B. pertussis, either nonopsonized or opsonized with IgA. In the infection model, decreased numbers of viable B. pertussis organisms in murine airways reflect protection against bacterial infection (36). Upon IgA opsonization, the numbers of viable B. pertussis organisms in lungs of Tg mice were significantly decreased compared to those in lungs of mice infected with nonopsonized bacteria. A decrease in colonization of lungs of NTg mice was also observed but was not significant (Fig. 4).

**DISCUSSION**

In spite of high vaccination coverage in developed countries, the incidence of B. pertussis infections appears to be rising (6). Research into the basis of immunity may lead to the development of more effective vaccines. In humans, infection is followed by the production of antibodies of several isotypes, including IgA (19, 37). In this study, anti-B. pertussis IgA was shown to be capable of inducing bactericidal effects by facilitating binding, phagocytosis, and killing of B. pertussis via the myeloid IgA receptor, FcαRI (CD89).

Human PMNL bound and phagocytosed IgA-opsonized B. pertussis, and both processes were inhibitable by blocking...
FccRI. This indicated that IgA-induced phagocytosis is largely mediated by FccRI. Tg mouse PMNL expressing human FccRI also exhibited potent IgA-mediated phagocytosis. FccRI-mediated phagocytosis resulted in enhanced killing of *B. pertussis* by PMNL. The enhanced phagocytosis of mouse PMNL relative to human cells (Fig. 2) is likely attributable to the treatment with G-CSF prior to mouse PMNL isolation, which is known to stimulate FccRI function (35). Although both Tg and NTg PMNL bound IgA-opsonized *B. pertussis*, binding and phagocytosis by Tg PMNL was clearly enhanced. *B. pertussis* binding to NTg PMNL is most likely mediated by *B. pertussis* virulence factors that interact directly with phagocyte receptors such as CR3 (20) and VLA-5 (11).

To prove that IgA-mediated effects were truly attributable to interaction with FccRI, experiments were performed with both IgA and CD89 BsAb. The advantage of CD89 BsAb is that they recognize FccRI outside its ligand-binding domain, which enables direct bacterial targeting to FccRI. All in vitro experiments were performed with both IgA and CD89 BsAb-opsonized bacteria, yielding similar results, which demonstrated that the IgA-mediated effects depend on FccRI triggering.

More importantly, IgA-mediated anti-*B. pertussis* activity was also observed in a murine pertussis infection model. Previously, high IgA titers in sera of human pertussis patients younger than 1 year of age were found to correlate with reduced duration of positive pertussis culture and PCR in throat samples (26). These findings pointed to bactericidal effects of anti-*B. pertussis* IgA in humans. Indeed, in our Tg mouse model, IgA opsonization of *B. pertussis* prior to infection resulted in increased bacterial clearance in lungs that was attributable to FccRI interaction.

The IgA used in our work was purified from immune sera of *B. pertussis* patients that were collected relatively soon after infection. Serum IgA consists mainly of IgA1, and in the upper respiratory tract IgA1 also represents the main antibody isotype (4). However, in contrast to serum IgA, mucosal secretory IgA is in a large part dimeric, containing the J chain and secretory component. Although secretory IgA is capable of interacting with FccRI, the types of functions initiated by serum and secretory IgAs may be different (4).

A recent study reported that serum opsonization of *B. pertussis* inhibited phagocytosis by PMNL compared to no opsonization (33). Our data indicate that purified IgA antibodies are able to increase PMNL binding and phagocytosis of *B. pertussis*. Our phagocytosis assay, however, differs from that used by Weingart et al. (33) in that we used PMNL in suspension rather than as adherent cells. Second, adenylate cyclase toxin was reported to be the virulence factor responsible for inhibition of opsonized *B. pertussis* phagocytosis (34). Our antibodies may (partly) consist of adenylate cyclase toxin-neutralizing antibodies, resulting in efficient phagocytosis in the present study.

A recent trial with pertussis vaccines in The Netherlands showed that boosting of 4-year-old children with the Dutch whole-cell pertussis vaccine induced anti-*B. pertussis*-specific serum IgA, in contrast to boosting with acellular vaccines (1). Our findings demonstrating IgA to be capable of inducing anti-*B. pertussis* activity may be important in the evaluation of vaccines. For years IgA has been considered to play a passive, “noninflammatory” role in immunity; by blocking microbial interaction with host tissue, it may prevent cell damage and inflammation. However, IgA proved to be very effective in inducing cellular immune functions via FccRI expressed on myeloid cells. A number of recent studies have already reported IgA-mediated phagocytosis of different microorganisms and tumor cells (7, 25, 27, 30). This study documents an important role for IgA in anti-*B. pertussis* activity and shows, for the first time, IgA-mediated bactericidal activity in vivo.
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