SUPPLEMENT ARTICLE

Mouse and Pig Models for Studies of Natural and Vaccine-Induced Immunity to *Bordetella pertussis*

Kingston H. G. Mills[1] and Volker Gerdts[2]

[1]School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Ireland; and [2]Vaccine and Infectious Disease Organization, International Vaccine Centre, University of Saskatchewan, Saskatoon, Canada

The increasing incidence of whooping cough in many developed countries has been linked with waning immunity induced after immunization with acellular pertussis (aP) vaccines. The rational design of an improved aP vaccine requires a full understanding of the mechanism of protective immunity and preclinical studies in animal models. Infection of mice and pigs with *Bordetella pertussis* has many features of the infection seen in humans and has already provided valuable information on the roles of innate and adaptive immune responses in protection. Recent findings in these models have already indicated that it may be possible to develop an improved aP vaccine based on a formulation that includes a Toll-like receptor agonist as an adjuvant.

Keywords. *Bordetella pertussis*; whooping cough; animal model; respiratory infection; vaccine; protective immunity; T cells; adjuvant.

Some of the greatest breakthroughs in modern medicine, including an understanding of the host immune control of infectious diseases have been made through studies in animal models. Although most animal models have limitations, the in-depth experiments involving knockout mice, cell transfers and experimental challenge with live pathogens could simply not be done in humans. Respiratory infection of mice, pigs and nonhuman primates with *Bordetella pertussis* has provided very useful models for studies on mechanisms of natural and vaccine-induced immunity to *B. pertussis* and in the rational design of improved pertussis vaccines.

BORDETELLA PERTUSSIS CHALLENGE MODELS IN MICE

A number of murine models of infection with *B. pertussis* have been developed with the aim of understanding the pathogenesis of infection, the mechanisms of immunity, and for development and efficacy testing of vaccines before their use in humans. The most frequently used model involves respiratory infection with live bacteria, delivered by intranasal or aerosol administration [1]. Although mice infected with *B. pertussis* do not develop the characteristic cough associated with the diseases in human infants and have lung pathologic findings different from those seen in humans, they do have many of the features of the human disease. The bacteria adhere to ciliated epithelium in the trachea and can bind to and invade macrophages in the lungs in both species. The disease is persistent, lasting 4–8 weeks in immunocompetent mice, and accompanied by leukocytosis and immune suppression. Recovery from respiratory infection is associated with the development of potent *B. pertussis*–specific immune responses and rapid clearance of the infection on rechallenge of convalescent mice [2].

Different laboratories employ either intranasal or aerosol delivery methods. The intranasal method is simpler, involving dropping a solution of bacteria on the nares of the mouse and waiting for it to inhale the bacteria. In contrast, the aerosol method requires a nebulizer, which delivers an aerosol of the bacteria, usually into a chamber, where the mice breathe in the bacteria over a period of 15–20 minutes. Although the amount

Correspondence: Kingston Mills, PhD, School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin 2, Ireland (kingston.mills@tcd.ie).

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of bacteria delivered is easier to control using the intranasal route, the aerosol challenge method delivers the bacteria deeper into the lungs and results in a very consistent bacterial load in each individual mouse exposed to an aerosol of the same bacterial culture. Furthermore, in this model the rate of bacterial clearance after aerosol challenge of mice immunized with different pertussis vaccines correlated with the efficacy of those vaccines in children [3].

Mice can also be infected with *B. pertussis* via the intracerebral route, which results in a lethal infection in the absence of an appropriate adaptive immune response. Although bearing no resemblance to human infection, the intracerebral challenge test, developed by Kendrick et al in the 1940s [4], was used for several decades as a quality test for new batches of whole-cell pertussis (wP). However, immunization of mice with the newer acellular pertussis (aP) generally does not prevent this lethal infection. Therefore, the intracerebral challenge or Kendrick test is now rarely used either for regulatory control of pertussis vaccines or for research on mechanism of immunity. In contrast, murine respiratory challenge models have been used with considerable success.

DEFINING MECHANISMS OF NATURAL IMMUNITY WITH CHALLENGE MODELS

Much of the early studies on natural immunity to *B. pertussis* generated by respiratory infection of mice focused on the role of antibodies (serum immunoglobulin [Ig] G and mucosal IgA). Passive transfer experiments with immune serum from convalescent mice showed that antibodies could transfer at least some protection [2]. However, serum IgG responses are slow to develop during primary infection of mice with *B. pertussis,* and it is likely that the main function of antibodies is to help prevent reinfection rather than to clear a primary infection [5]. More recently the focus has turned to the role of T cells and innate immunity. Macrophages, dendritic cells, neutrophils, natural killer cells, and γδ T cells infiltrate the lungs during the first 2–3 weeks of infection [5]. This is followed by infiltration of CD4 and CD8 T cells. Interleukin 10–secreting regulatory T (Treg) cells and $Foxp3^+$ Treg cells are induced or recruited into the lungs during infection with *B. pertussis,* simultaneously with interleukin 17 (IL-17)–secreting CD4 T cells (T-helper [Th] 17 cells) and interferon (IFN) γ–secreting CD4 T cells (Th1 cells) [6–8]. It seems that Treg cells suppress protective immune responses during acute infection, which may in part explain the persistence of the infection. Nevertheless, effector Th1 and Th17 cells eventually help clear the infection by promoting the recruitment and activation of neutrophils and macrophages [9–11].

Studies with severe combined immunodeficient mice lacking T or B cells or athymic mice showed that mice lacking just T cells either died from infection or developed a chronic infection that did not clear for the lifetime of the mouse [5, 9, 10, 12]. These studies provided some of the first clear evidence that cellular immunity played a critical role in clearance of a primary infection with this bacterium. Follow-up studies using mice defective in individual cytokines identified critical protective roles for IFN-γ and IL-17A [9–11]. Finally, depletion experiments with antibodies directed against particular cell types confirmed the roles for CD4 T cells, natural killer cells, neutrophils, and macrophages in clearance of *B. pertussis* from the respiratory tract [12–14].

USING MURINE RESPIRATORY CHALLENGE MODELS TO STUDY VACCINE-INDUCED IMMUNITY

Much of the focus in the clinical trials in the 1990s that compared aP with wP vaccines was on the role of antibodies. Although the results of household contact studies suggested that children with high antibody responses to pertussis toxin (PT) and pertactin were less likely to develop pertussis [15, 16], most studies failed to provide convincing evidence that antibodies against ≥1 antigen conferred protective immunity. Indeed, studies in children revealed that antibodies and protective efficacy declined rapidly after immunization with aP vaccine [17]. Complementary studies in the mouse model showed that it was possible to passively transfer protection by using high-titer antibodies generated against individual antigens, in particular PT and pertactin, but to a lesser extent filamentous hemagglutinin and fimbrae [3]. However, mouse studies also showed that antibody responses declined rapidly after immunization with aP and wP vaccines and that a good level of protection persisted in mice immunized with wP vaccine after decline of antibody response to undetectable levels [18]. Conversely, T-cell responses were more persistent, and memory T-helper cells seem important for long-term immunity [18].

Studies on vaccine-induced cellular immunity in the murine respiratory challenge model revealed that, analogous to natural infection, immunization with wP vaccines was associated with the induction of strong Th1-type responses, whereas aP preferentially induced Th2 cells [3, 9, 10, 19]. This finding is largely consistent with the Th1- and Th2-dominated responses observed in humans with wP and aP vaccines, respectively, especially after repeated booster doses [20–22]. After the discovery of Th17 cells, which are pathogenic in many autoimmune diseases but protective against fungi and extracellular bacteria, it was demonstrated that both aP and wP also induce this CD4 T-cell subtype [11, 23]. Indeed, recent studies using IL-17A– and interleukin 4–defective mice have revealed that Th17 but not Th2 cells confer protection with aP in mice, whereas IFN-γ–secreting Th1 cells are critical for protective immunity induced with wP [11].

MOUSE MODELS FOR THE RATIONAL DESIGN OF IMPROVED aP VACCINES

The current aP vaccine was designed empirically, with some information on protective antigens but little knowledge of the

mechanism of protective immunity or the importance of the adjuvant. Alum was chosen as the adjuvant for aP largely for historical reasons and because it would have less obstacles to licensure by the regulatory agencies and was already used for a range of pediatric vaccines, including those for diphtheria and tetanus. Studies in mice have shown that alum tends to promote Th2- rather than Th1-type responses and IgG1 rather than IgG2a, whereas several new-generation adjuvants, including Toll-like receptor (TLR) agonists, can generate Th1 and IgG2a responses.

Recent studies have shown that the immune response induced by aP can be switched from Th2 to Th1 by changing adjuvants from alum to CpG, with both adjuvants also inducing Th17 responses [11]. Significantly, the switch from alum to the Th1-promoting TLR agonist significantly enhanced the efficacy of the aP vaccine in the murine model [11]. A complementary study showed that adding CpG to aP that was already absorbed to alum also enhanced the efficacy of the vaccine in mice [24]. This positive influence of the Th1-inducing adjuvant is not confined to TLR agonists, because similar results were observed when recombinant interleukin 12 was used as the adjuvant [25]. Interleukin 12 promotes the induction of Th1 cells and. when added to an experimental aP vaccine, enhanced the rate of bacterial clearance in immunized mice after aerosol challenge with *B. pertussis* [25]. Furthermore, a combination adjuvant consisting of CpG oligodeoxynucleotides, innate defense regulator peptides, and polyphosphazenes provided protection against respiratory infection with *B. pertussis* in neonatal and adult mice [26]. A single intranasal immunization with either the genetically detoxified PT and pertactin or filamentous hemagglutinin with this combined adjuvant induced potent serum antibody (IgG1 and IgG2a) and mucosal IgA responses in neonatal mice, which were unaffected by the presence of maternal antibodies [27]. The nasally delivered attenuated *B. pertussis* vaccine BPZE1 is also highly protective in mice [28], and it has the advantage of inducing mucosal as well as cellular immunity. Although severe local and systemic adverse events are observed after vaccination with wP vaccines, these less like to occur with aP, whether given with alum or TLR agonists as the adjuvant.

PIG MODEL

Pigs are a natural host to *Bordetella bronchiseptica* and under experimental conditions can also be infected with *B. pertussis* and *Bordetella parapertussis* [29, 30]. Infected piglets display a wide range of respiratory symptoms, including fever, nasal discharge, nonparoxysmal coughing, and breathing difficulties resulting in severe bronchopneumonia, which in some cases was combined with a fibrinous pleuritis. *B. pertussis* can be found within airways adhering to the epithelial lining or phagocytosed by macrophages and neutrophils. Hypoglycemia and lymphocytosis were found in infected animals. Viable bacteria can be reisolated from bronchoalveolar lavage specimens and lung lesions for >10 days after infection [31]. Interestingly, only younger piglets are susceptible to the disease, and at 5 weeks of age pigs become completely resistant to the infection.

Protection in older pigs is associated with innate immune defenses in the respiratory tract, in particular host defense peptides, also called antimicrobial peptides. For example, expression of porcine β-defensin 1 is essential for protection against the disease [31]. Furthermore, adaptive immune responses in pigs, such as systemic and mucosal IgG and IgA, can be detected in response to immunization and infection. Thus, this model can be used to assess novel vaccine candidates in preclinical settings. Indeed, formulation of pertussis toxoid with a novel adjuvant combination consisting of CpG ODN, polyphosphazenes, and innate defense regulator peptides induced immune responses that lasted for more than a year after immunization, with a shift toward a Th1-type response [27]. The vaccine was highly effective in 3-day-old piglets, with an early onset of immunity. Interestingly, the vaccine was not affected by the presence of maternal antibodies [32].

Pigs are an important large animal species often used for vaccine research. Their immune system closely resembles that of humans. They are outbred, and both systemic and mucosal immune compartments are easily accessible. The immunologic toolbox has improved greatly over the last few years; all major markers are available for use in porcine cells. Notably, the major dendritic cell populations can be found in pigs with TLR distributions and responsiveness to TLR stimulation similar to that in humans and in contrast to that of mice. In addition, the Th17 cells of pigs have functions similar to their human counterparts [33]. Thus, pigs are an excellent model for respiratory diseases in humans, including influenza and pertussis. Furthermore, owing to the ability to sample both colostrum and milk and to exchange litters after birth, pigs are an excellent model for studying the effectiveness of maternal immunization against infectious diseases in the offspring. Indeed, maternal immunization of pigs resulted in complete protection against challenge infection with *B. pertussis* [34].

CONCLUSIONS AND FUTURE PERSPECTIVE

Recent studies in mice and pigs have provided convincing evidence that it may be possible to improve the efficacy of aP vaccines in children by changing the adjuvant from alum to a TLR agonist. However, the practicalities of implementing such a change are considerable, because the current aP vaccine is administered to children as part of a combination vaccine against 7 or 8 infectious diseases. Unbundling the combination or adding another adjuvant to the existing vaccine may be logistically difficult but not impossible. Initially, studies should focus on developing a Th1-promoting vaccine containing only pertussis components for booster immunization. It would be useful to begin this work in vaccines targeted for adolescents

and adults, who will benefit from pertussis-only vaccines, before progressing to combination vaccines in young children.

Notes

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