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IL-10 Induction by *Bordetella parapertussis* Limits a Protective IFN-γ Response

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Abstract

Bordetella parapertussis causes the prolonged coughing illness known as pertussis or whooping cough, persisting for weeks within the respiratory tracts of infected hosts but inducing a very poor T cell response relative to that induced by *Bordetella pertussis*, the more common cause of pertussis. In this study, we examine the contributions of cytokines involved in the clearance of *B. parapertussis* and immunomodulation that delays effective clearance. The slow elimination of this pathogen from the respiratory tracts of mice coincides with the gradual accumulation of $CD4^+$ T cells in the lungs and *B. parapertussis*-responsive IFN-γ–producing cells in the spleen. IFN-γ–deficient mice were defective in the accumulation of leukocytes in lungs and in clearance of *B. parapertussis* from the lungs. In vitro *B. parapertussis*-stimulated macrophages produced IL-10, which inhibited the generation of the IFN-γ response that is required for protection in vivo. As compared with wild-type mice, IL-10–deficient mice produced significantly higher levels of IFN-γ, had higher numbers of leukocytes accumulated in the lungs, and cleared *B. parapertussis* more rapidly. Together, these data indicate that *B. parapertussis* induces the production of IL-10, which facilitates its persistence within infected hosts by limiting a protective IFN-γ response.

Despite the intricate defense mechanisms used by the mammalian immune system, the threat of disease is ongoing and acts as a stark reminder of the fact that successful microbial pathogens appear to have developed ways of manipulating and evading them. For example, several pathogens are known to promote the production of factors such as IL-10, an anti-inflammatory cytokine and an important regulator of the host immune response, to avoid induction of the host inflammatory response (reviewed in (1, 2)). This cytokine is produced

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by APCs, such as macrophages and dendritic cells, and results in a T cell response skewed toward a Th2 or T_{reg} phenotype (3). IL-10 is also produced in large quantities by T_{reg} cells and serves as a negative regulator of both Th1 and Th2 responses (4). Importantly, IL-10 inhibits the production of proinflammatory cytokines such as IFN-γ (5, 6). This inhibitory effect is mediated by the suppressive effects of IL-10 on APCs as well as by the upregulation of *SOCS1*, the gene responsible for the majority of the physiological inhibition of IFN-γ–induced signaling (7, 8).

IFN-γ, which is largely produced by NK cells and activated T cells, contributes to protective immunity against microbial pathogens in part by enhancing both the recruitment of leukocytes to the site of infection and the activation of these cells (9–11). IFN-γ also upregulates the expression of MHC molecules, in addition to other costimulatory molecules, on APCs (12, 13) and drives the differentiation of naive T cells to Th1 cells (14). Although this cytokine is protective against a wide range of pathogens (15, 16), excessive production can lead to severe, uncontrolled inflammation and subsequent damage to host tissues (17, 18). IL-10–mediated inhibition of IFN-γ is crucial for protection against immune-mediated inflammatory disorders such as colitis and experimental autoimmune encephalitis (19–21). Notably, several bacterial pathogens dampen the IFN-γ response, often limiting the protection conferred by this proinflammatory cytokine (22–26). For instance, the inhibition of IFN-γ production by low calcium response toxin enhances the lethality of *Yersinia pestis* infection (27). Furthermore, some pathogens can modulate specific aspects of IFN-γ–induced signaling pathways to optimize their interactions with the host. For example, *Chlamydia* species inhibit IFN-γ–mediated MHC class II upregulation, limiting the capacity of $CD4^+$ T cells to respond to the infection (28).

Bordetella pertussis and *Bordetella parapertussis* are the etiologic agents of whooping cough in humans, which is a severe coughing illness that is re-emerging in even highly vaccinated populations (29–33). These bacteria spread between hosts via cough-produced aerosolized droplets, and the ability to persist within the host respiratory tract increases the amount of time during which the bacteria are shed, thereby enhancing the opportunity for transmission. Previous studies have addressed how *B. pertussis* persists within its host; pertussis toxin (PTX) prevents the migration of neutrophils to the lungs, allowing *B. pertussis* to grow to higher numbers in both naive and immune hosts (34, 35). Although not as well studied as *B. pertussis, B. parapertussis* behaves similarly in a mouse model of infection despite notable differences from *B. pertussis*, such as the absence of PTX. Inefficient stimulation of proinflammatory TLR4 responses appear to allow it to grow rapidly over the first few days, after which point bacterial numbers slowly decline over a month or more (36). This persistence led us to investigate how *B. parapertussis* modulates immune responses to extend its survival within infected hosts.

Both *B. pertussis* and *B. bronchiseptica* induce IL-10 as a means of limiting the host immune response to allow for persistence within the host, although these bacteria use different subsets of virulence factors not expressed by *B. parapertussis* or by one another, indicating that the mechanisms by which these pathogens modulate host immunity may differ between species. *B. bronchiseptica* induces IL-10 via the type III secretion system (25), whereas *B. pertussis* stimulates the production of this cytokine through the synergistic

action of adenylate cyclase toxin and LPS (37). *B. parapertussis*, although closely related to these pathogens, differs greatly in its expression of virulence factors. For instance, the LPS of *B. parapertussis* is significantly less stimulatory of TLR4 than that of either *B. pertussis* or *B. bronchiseptica*, and human-adapted *B. parapertussis* strains do not express a type III secretion system (25, 38, 39). In our studies, we evaluate whether the induction of IL-10 contributes to the persistence of *B. parapertussis* in the host respiratory tract.

We have previously documented that $CD4^+$ T cells are important for the elimination of *B. parapertussis* from the lower respiratory tract (40). In this study, we show that the reduction of bacterial numbers corresponds to an accumulation of $CD4^+$ T cells in the lungs, as well as to the production of IFN-γ by T cells. Furthermore, we show that this proinflammatory cytokine is required for both the efficient recruitment of leukocytes and the clearance of *B. parapertussis* from the lungs. However, the IFN-γ response generated against *B. parapertussis* is delayed by the production of IL-10, which decreases leukocyte accumulation and delays the clearance of *B. parapertussis* from the lungs. Together, these data suggest that the induction of an IL-10 response, which subsequently limits protective IFN-γ production, allows *B. parapertussis* to persist within the host respiratory tract.

Materials and Methods

Bacterial strains and growth

B. parapertussis strain 12822 was isolated from German clinical trials and has previously been described (41). *B. parapertussis* strain 12822G is an isogenic gentamicin-resistant derivative of 12822 (40). Bacteria were maintained on Bordet−Gengou agar (Difco, Albany, NY) containing 10% defibrinated sheep blood (Hema Resources, Aurora, OR) and appropriate antibiotics. Liquid cultures of bacteria were grown at 37°C overnight on a roller drum to midlog phase (an OD of ~0.3) in Stainer-Scholte broth. Liquid cultures were incubated at 70°C for 30 min to generate preparations of heat-killed bacteria. These cultures were plated before and after incubation to determine the number of viable bacteria present.

Inoculation of mice

C57BL/6, TCRβδ$^{-/-}$ (B6.129P2-*Tcrb*^{tm1Mom}*Tcrd*^{tm1Mom}/*J*), IFN-γ$^{-/-}$ (B6.129S7-*Ifng*^{tm1Ts}/*J*), and IL-10$^{-/-}$ (B6.129P2-*Il10*^{tm1Cgn}/*J*) mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and bred in our *Bordetella*-free, specific pathogen-free facilities at The Pennsylvania State University. Bacteria grown overnight in liquid culture were diluted in PBS to ~1×10^7 CFU/ml. Groups of 4- to 6-wk-old mice were lightly sedated with 5% isoflurane in oxygen and inoculated by pipetting 50 μl of the inoculum (5×10^5 CFU) onto the external nares. Heat-killed bacteria were diluted to 10^9 CFU/ml and delivered by pipetting 50 μl (5×10^7 CFU) onto the external nares. All of the protocols were reviewed by the university's Institutional Animal Care and Use Committee, and all of the animals were handled in accordance with institutional guidelines.

Quantification of bacteria and leukocytes in the lungs

To quantify bacteria, lungs were excised on day 0, 3, 7, 14, or 28 post-inoculation and homogenized in 1 ml of PBS, and the homogenate was serially diluted and plated onto

Bordet–Gengou agar plates. CFUs were counted after incubating for 3 d at 37°C. To quantify leukocytes, bronchoalveolar lavage fluid (BALF) was collected from *B. parapertussis*-infected mice on day 0 (uninfected mice), 3, 7, 14, or 28 postinoculation. RBCs were lysed by treatment with 0.83% ammonium chloride as previously described (18). Leukocytes were counted on a hemocytometer to quantify total numbers in the BALF. Aliquots of cells were then stained with FITC-labeled anti–Ly-6G (eBioscience, San Diego, CA) for neutrophils, FITC-labeled anti-F4/80 (eBioscience) for macrophages, or FITC-labeled anti-CD4 (eBioscience) for $CD4^+$ T cells, and the percentage of FITC-positive cells was multiplied by the total number of leukocytes to calculate the numbers of neutrophils, macrophages, and $CD4^+$ T cells, respectively.

Splenocyte restimulations

Restimulation of splenocytes was performed as previously described (18). Briefly, splenocytes were isolated by homogenizing spleens through a wire sieve, spinning at 400 × *g* for 5 min at 4°C, lysing the RBCs with 0.83% ammonium chloride, and then washing the cells with DMEM (HyClone, Logan, UT). Cells were resuspended in DMEM supplemented with 10% FCS (HyClone), 1 mM sodium pyruvate (HyClone), and 100 μg/ml penicillin and streptomycin (HyClone) at a concentration of 1×10^7cells/ml. Two hundred microliters of this suspension (2×10^6 cells) were placed into each well of a 96-well plate. Splenocytes were stimulated with medium alone or 1×10^7 CFU of heat-killed (HK) *B. parapertussis* (multiplicity of infection (MOI) of 5). After 3 d, the supernatant was collected and analyzed for IFN-γ and IL-10 production as specified by the manufacturers of ELISA kits (R&D Systems, Minneapolis, MN).

Stimulation of macrophage cell lines

The murine macrophage cell line MH-S was obtained from American Type Culture Collection (Manassas, VA) and cultured in RPMI 1640 supplemented with 10% FBS, penicillin, and streptomycin. Cells were placed in 96-well plates with ~10^5 cells/well and stimulated with either live *B. parapertussis* or HK *B. parapertussis* at MOIs of 1, 10, or 100 or 1 or 100 ng/well of purified *B. parapertussis* LPS to equal a total volume of 100 μl/well (19). HK *B. parapertussis* was made by heating cultures at 65°C for 30 min. Proteinase K was added to cultures at a concentration of 50 μg/ml for 30 min at 37°C. Both trypsin and RNase were added at a concentration of 200 μg/ml at 37°C for 10 and 30 min, respectively. Cell culture supernatants were collected after 24 h, and the concentration of IL-10 was measured via ELISA. These results were also reproduced using J774 cells, a murine peritoneal macrophage cell line.

Intracellular cytokine staining

Peritoneal exudate cells were analyzed by flow cytometry for intracellular IL-10. Briefly, cells were collected, RBCs were lysed with 0.83% ammonium chloride solution, and leukocytes were resuspended in DMEM before being counted on a hemocytometer. Approximately 2×10^6 cells were incubated at 37°C in 5% CO_2 with 1×10^7 CFU of HK *B. parapertussis* and brefeldin A for 12 h. Cells were stained with a FITC-labeled surface marker for macrophages (F4/80), fixed, permeabilized, and stained with PE-labeled IL-10

Abs according to the manufacturer's protocol (eBioscience). Cells were analyzed by flow cytometry for the quantification of double-positive cells.

Histological examination

Lungs were removed and inflated with 10% neutral buffered formalin, embedded in paraffin, and sectioned. Slides were prepared and stained with H&E, and an assessment of microscopic lesions was made by one of the authors (M.J.K.), who is experienced in rodent pathology and was blinded to the experimental treatments. Descriptive evaluations of the lesions were recorded, and lung lesions were graded by using a scale of 0–4. Sections with no lesions and no inflammation were given a score of 0, a score of 1 indicated slight inflammation with few or scattered lesions and <10% of lung fields affected, a score of 2 indicated mild lesions with 10–20% of lung fields affected, a score of 3 indicated moderate lesions with 20–30% of the lung fields affected, and those given a score of 4 were characterized by extensive lesions, marked inflammation, and 31–50% of the lung affected. Plus marks indicated scores with a severity that fell between categories. Photographs were taken at ×100 magnification with an Olympus (Center Valley, PA) BH-2 light microscope fitted with a Nikon (Melville, NY) digital camera (DXM1200F).

Statistical analysis

Three to four mice were used per group per individual time point in each experiment. The mean for each group of mice ± SD (error bars) was determined for CFU, leukocytes, and cytokines. Two-tailed, unpaired Student *t* tests were used to determine statistical significance between groups. All of the experiments were performed at least twice with similar results, and figures shown are representative of one experiment. $p < 0.05$ were taken to be statistically significant.

Results

T cells required for the clearance of *B. parapertussis* are slowly recruited to the lungs

B. parapertussis persists in the respiratory tract of its host for a month or more but is unlikely to do so by the same mechanism as *B. pertussis* because it lacks PTX. To study the mechanism by which *B. parapertussis* may manipulate host immune responses, we first examined the kinetics of the generation of responses that are involved in protection. C57BL/6 mice were inoculated with *B. parapertussis* and sacrificed on day 0, 3, 7, 14, or 28 postinoculation to quantify bacterial numbers in the lungs and $CD4^+$ T cell numbers in the BALF, because these cells are essential to the clearance of *B. parapertussis* (40). *B. parapertussis* numbers peaked at ~4×10^6 CFU on day 3 postinoculation and slowly declined thereafter until bacteria were nearly undetectable by day 28 postinoculation (Fig. 1). $CD4^+$ T cells were almost undetectable on days 0 and 3 postinoculation but had increased to ~3×10^3 cells/ml of BALF by days 7 and 14 postinoculation and to 2×10^4 cells/ml of *B. parapertussis* correlates with the slow accumulation of $CD4^+$ T cells in the BALF, although they do not define the numbers of T cells in tissues or nodes or the function of those cells.

T cells produce IFN-γ, which facilitates the clearance of *B. parapertussis*

IFN-γ is a proinflammatory cytokine required for protection against the closely related pathogens *B. pertussis* (42, 43) and *B. bronchiseptica* (25). Because activated T cells are a major source of IFN-γ, we examined the IFN-γ response against *B. parapertussis* in wild-type and T cell-deficient mice. First, we investigated the presence of IFN-γ in the lung homogenates during infection and found the levels to be very low. Therefore, we examined the systemic cytokine response generated against *B. parapertussis* through restimulation of splenocytes over the course of infection, which provided a more robust representation of the Ag-specific cytokine response. C57BL/6 and TCRβδ$^{-/-}$ mice were inoculated with *B. parapertussis*, and their spleens excised for ex vivo assessment of IFN-γ production on days 0, 7, 14, and 28 postinoculation. IFN-γ was produced at low levels (<50 pg/ml) by media-stimulated splenocytes, which serves as the unstimulated control to determine background cytokine levels, from either mouse strain (Fig. 2). Upon stimulation with HK *B. parapertussis*, splenocytes from wild-type mice produced IFN-γ, which peaked on days 7 and 28 postinoculation at concentrations of 1000 and 200 pg/ml, respectively, with low levels observed between these time points (Fig. 2). Similar results were observed upon examination of lung homogenate cytokines in C57BL/6 mice during infection (data not shown). HK *B. parapertussis* stimulated splenocytes from T cell-deficient mice produced low levels of IFN-γ (<50 pg/ml) at all time points (Fig. 2), indicating that T cells are required for the efficient production of this cytokine in response to *B. parapertussis.*

We hypothesized that IFN-γ contributes to the clearance of B. parapertussis from the lungs. To test this, C57BL/6 and IFN-γ $^{-/-}$ mice were inoculated with *B. parapertussis* and sacrificed on day 0, 3, 7, 14, or 28 postinoculation for the enumeration of bacterial numbers in the lungs. In wild-type mice, *B. parapertussis* numbers peaked at ~5×10^6 CFU on day 3 postinoculation and declined thereafter until nearly undetectable by day 28 postinoculation (Fig. 3). Bacterial numbers in the lungs of IFN-γ $^{-/-}$ mice were similar to those of wild-type mice on days 3 and 7 postinoculation. However, the lungs of IFN-γ$^{-/-}$ mice harbored ~100-fold more bacteria as compared with those of wild-type mice on days 14 and 28 postinoculation (Fig. 3). Overall, these data indicate that IFN-γ is required for the efficient elimination of *B. parapertussis* from the lungs of mice.

IFN-γ is required for the efficient recruitment of leukocytes to the lungs upon *B. parapertussis* infection

IFN-γ can mediate the recruitment of leukocytes to the site of infection by inducing the production of chemokines by immune cells (44, 45). To assess the role of IFN-γ in the recruitment of leukocytes during *B. parapertussis* infection, C57BL/6 and IFN-γ$^{-/-}$ mice were inoculated with *B. parapertussis* and sacrificed on day 3, 7, 14, or 28 for BALF leukocyte enumeration. In wild-type mice, few leukocytes were recovered from the BALF by day 3 postinoculation (~5×10^5). Leukocyte numbers rose to peak levels on day 7 postinoculation (~2×10^6) and declined thereafter (Fig. 4*A*). Leukocyte numbers in the BALF of IFN-γ$^{-/-}$ mice were also low on day 3 postinoculation (~5×10^5) but remained low even on day 7 postinoculation (~8×10^5) (Fig. 4*A*). Similarly, more neutrophils and macrophages were recovered from the lungs of wild-type mice as compared with those of IFN-γ$^{-/-}$ mice (Fig. 4*B*, 4*C*), although IFN-γ did not appear to affect the accumulation of T

cells (data not shown). Thus, IFN-γ contributes to the efficient recruitment of leukocytes to the lungs in response to *B. parapertussis* infection.

IL-10 is produced by macrophages in response to *B. parapertussis*

IFN-γ is important for the efficient clearance of *B. parapertussis* from the lungs, but its production can be modulated by IL-10 (46), an anti-inflammatory cytokine known to be induced by other Bordetella species (25, 37). Therefore, we measured IL-10 production in response to *B. parapertussis* infection and assessed the cell type responsible for the production of this cytokine. C57BL/6 and TCRβδ$^{-/-}$ mice were inoculated with *B. parapertussis* and sacrificed on day 0, 3, 7, 14, or 28 postinoculation. Splenocytes were isolated and stimulated with HK *B. parapertussis* to assess IL-10 production by these cells. IL-10 was nearly undetectable in the supernatants of unstimulated splenocytes at any time point. In contrast, IL-10 was produced by HK *B. parapertussis* stimulated splenocytes from wild-type and T cell-deficient mice (200 and 350 pg/ml, respectively) on day 3 postinoculation, whereas lower levels (<50 pg/ml) were observed in the supernatants of stimulated splenocytes from mice that had been infected for 7 d (Fig. 5*A*). After day 7 postinoculation, IL-10 was produced in large amounts, but wild-type splenocytes produced significantly more than T cell-deficient splenocytes (day 14, 700 and 400 pg/ml; day 28, 4000 and 600 pg/ml, respectively). Together, these data indicate that some non-T cell produces IL-10 early in response to *B. parapertussis*, whereas T cells produce larger amounts of this cytokine after the first week of infection.

Because macrophages provide a first line of defense against infection and can produce IL-10, we examined whether *B. parapertussis* induces the production of IL-10 by these cells. Peritoneal exudate cells isolated from wild-type mice were incubated with HK *B. parapertussis* and intracellularly stained to assess IL-10 production induced upon primary Ag exposure. Although only 3% of macrophages stimulated with the media control were positive for intracellular IL-10, ~40% of macrophages stimulated with *B. parapertussis* were positive for this cytokine after 12 h (Fig. 5*B–F*), indicating that macrophages produce IL-10 in response to *B. parapertussis*.

MH-S cells, a murine alveolar macrophage cell line, were then stimulated with live *B. parapertussis*, HK *B. parapertussis*, or purified *B. parapertussis* LPS. Live bacteria induced dose-dependent production of IL-10, with an MOI of 100 eliciting ~50 pg/ml of IL-10 (Fig. 6*A*). In contrast, an equivalent number of heat-killed bacteria induced much lower levels of IL-10 at a concentration of only 20 pg/ml (Fig. 6*A*). Therefore, live *B. parapertussis* and to a lesser extent HK *B. parapertussis* induce the production of IL-10 by macrophages. *B. parapertussis* LPS (1 and 100 ng/well) did not induce levels of IL-10 that were measurably different from those induced by PBS alone (data not shown). In addition, when live *B. parapertussis* was treated with formalin, trypsin, or proteinase K prior to stimulation of the MH-S cells, IL-10 production was markedly decreased to only 13 pg/ml or less at an MOI of 100 (Fig. 6*A*). To further understand the mechanism by which *B. parapertussis* induces IL-10 production in macrophage cells, MH-S cells were also stimulated with culture supernatants. Macrophage cells treated with *B. parapertussis* culture supernatants produced IL-10 at levels comparable to those of live *B. parapertussis*-stimulated cells, although this

effect was abrogated when supernatants were treated with formalin, trypsin, or proteinase K (Fig. 6*B*). Strikingly, when *B. parapertussis* culture supernatants were treated with RNase, IL-10 production was only slightly decreased as compared with live *B. parapertussis* or *B. parapertussis* culture supernatants alone (Fig. 6*B*). On the basis of these data, *B. parapertussis* appears to secrete a heat-labile protein factor that induces the production of IL-10 by macrophages.

IL-10 inhibits IFN-γ production and the resulting inflammatory response to *B. parapertussis*

To investigate the possibility that IL-10 limits the IFN-γ–mediated leukocyte recruitment important for the clearance of *B. parapertussis* infection, C57BL/6 and IL-10$^{-/-}$ mice were inoculated with *B. parapertussis* and sacrificed on day 0, 3, 7, or 14 postinoculation for the quantification of leukocytes in the BALF. Again, the peak of leukocyte accumulation in the lungs occurred around day 7 postinoculation in wild-type mice, although this peak was significantly higher in the lungs of IL-10$^{-/-}$ mice (Fig. 7*A*). Similarly, more neutrophils were recovered from the lungs of IL-10$^{-/-}$ mice (6×10^5) compared with those of wild-type mice (1.5×10^5) on day 7 postinoculation (Fig. 7*B*), indicating that IL-10 does limit the accumulation of neutrophils in the lungs during *B. parapertussis* infection. In addition, histological examination of wild-type versus IL-10–deficient lungs was performed to further assess the inflammatory infiltrate induced by *B. parapertussis* infection. Little inflammation was observed in the lungs of naive wild-type (Fig. 7*C*) or IL-10–deficient mice (Fig. 7*D*). By day 3 postinoculation, there were few signs of inflammation in the lungs of wild-type mice (Fig. 7*E*), but foci of inflammation, largely composed of neutrophils, were observed in the lungs of IL-10$^{-/-}$ mice (Fig. 7*F*). The lungs of wild-type mice contained numerous mild lesions by day 7 postinoculation (Fig. 7*G*), whereas those of IL-10$^{-/-}$ mice presented with lesions in addition to regions of pneumonia and leukocyte infiltration (Fig. 7*H*). Together, these data indicate that IL-10 inhibits the inflammatory response against *B. parapertussis* infection.

IL-10 inhibits the clearance of *B. parapertussis* from the respiratory tract

If inhibiting protective leukocyte influx via IL-10 induction is a strategy used by *B. parapertussis* to persist in its host, then we would expect that this pathogen would be more rapidly eliminated from IL-10-deficient hosts. To test this, C57BL/6 and IL-10$^{-/-}$ mice were inoculated with *B. parapertussis* and sacrificed on days 0, 3, 7, 14, or 28 postinoculation for the quantification of bacterial numbers in the lungs. *B. parapertussis* numbers peaked on day 3 (4×10^6 CFU) in the lungs of wild-type mice and were reduced to 3×10^4 and <100 CFU by days 14 and 28, respectively (Fig. 8). In the lungs of IL-10$^{-/-}$ mice, bacterial numbers also peaked at 4×10^6 CFU on day 3 postinoculation but were reduced to <100 CFU by day 14 and were undetectable by day 28 postinoculation (Fig. 8). Therefore, the 100-fold higher CFU in C57BL/6 versus IL-10$^{-/-}$ mice indicates that IL-10 delays the elimination of *B. parapertussis* from murine lungs.

Because an innate IL-10 response could skew the T cell response toward a Th2 or T_{reg} phenotype, we assessed the effect of IL-10 on the IFN-γ response generated against *B. parapertussis* (47). The amounts of IFN-γ produced by splenocytes from C57BL/6 versus

IL-10$^{-/-}$ mice in response to *B. parapertussis* after 0, 7, and 28 d of infection were compared. Unstimulated splenocytes produced <50 pg/ml of IFN-γ. Similarly, *B. parapertussis*-stimulated splenocytes from naive mice produced low levels of this cytokine. *B. parapertussis*-stimulated splenocytes from C57BL/6 mice infected with *B. parapertussis* for 7 or 28 d produced ~1750 and 250 pg of IFN-γ, respectively (Fig. 9), whereas those from IL-10$^{-/-}$ mice produced ~12,500 and 6000 pg of IFN-γ, respectively, at these times (Fig. 9). Similar results were also observed upon examination of lung homogenate cytokines (data not shown). Overall, these data indicate that *B. parapertussis* induces much higher IFN-γ production in the absence of IL-10.

Discussion

Acute pathogens have a limited opportunity to transmit to new hosts during short infectious periods, providing a strong selective advantage to those that can develop mechanisms to prolong infection. Furthermore, many pathogens, including the bordetellae, are capable of modulating aspects of the immune system to promote their persistence within hosts. Because IL-10 is an anti-inflammatory cytokine that significantly dampens critical immune effectors known to be involved in bacterial clearance, there should be strong selective advantage to pathogens that have evolved mechanisms by which they can influence the production of this cytokine to avoid immune-mediated clearance. There are examples of both microbial pathogens and commensals that induce IL-10 production by tissue resident macrophages and dendritic cells as a means of dampening host immune responses (48).

Interestingly, IL-10–deficient mice clear both *B. bronchiseptica* and *B. parapertussis* from their respiratory tracts more rapidly than wild-type mice (25; Fig. 8), although the absence of IL-10 does not appear to affect the course of *B. pertussis* infection (data not shown), an observation likely due to the expression of PTX by this species. During *B. bronchiseptica* or *B. parapertussis* infection, IL-10 limits the IFN-γ–mediated recruitment of leukocytes to the lungs. Consequently, in the absence of IL-10, a stronger inflammatory response may result in more efficient clearance of these pathogens. Although more IFN-γ could also be produced during *B. pertussis* infection in IL-10–deficient hosts, the expression of PTX inhibits the recruitment of phagocytic cells (35, 49, 50) and may, therefore, overcome the inflammatory effects of increased IFN-γ on bacterial clearance.

The protective immune response against infection by *B. parapertussis* should be considered when creating the next generation of whooping cough vaccines. Although the incidence of whooping cough dropped upon the introduction of whole-cell whooping cough vaccines in the 1940s and 1950s (51, 52), the switch from whole-cell to acellular vaccines has roughly correlated with the recent resurgence of whooping cough. Importantly, neither vaccine is very effective against *B. parapertussis*, with the acellular vaccine being even less effective than whole-cell vaccines (53, 54). Whole-cell vaccines stimulate a Th1-skewed response characterized by large amounts of IFN-γ, but current acellular vaccines induce a Th2-skewed T cell response with little *Bordetella*-specific IFN-γ (53–55). Because this cytokine is now known to be protective against both causative agents of whooping cough, *B. pertussis* (43) and *B. parapertussis* (Fig. 2), it seems likely that modifying vaccines to induce more of a Th1 response could help to combat the resurgence of this disease.

Although *B. parapertussis* is able to cause the same respiratory illness as *B. pertussis* despite the absence of PTX, little is known regarding the virulence factors that are important to its pathogenesis. Recent work has illuminated the importance of O Ag, which is not expressed by *B. pertussis*, in *B. parapertussis* pathogenesis. We have previously reported that O Ag enables *B. parapertussis* to avoid *B. pertussis*-induced Ab responses (56), potentially providing an explanation as to how *B. parapertussis* causes disease in *B. pertussis*-immunized individuals (57). Also, O Ag enhances the evasion of complement-mediated control by this pathogen (58). Despite its protective role, the fact that O Ag is a component of LPS makes it an unlikely candidate to be included in future vaccines. Although it is unclear which *B. parapertussis* protein toxins and/or adhesins could be effectively used in immunizations, our observation that IL-10 induction facilitates its persistence by modulating IFN-γ provides important insight into the pathogenesis of *B. parapertussis* disease and the type of immune response required for efficient protection against this respiratory pathogen.

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Abbreviations used

BALF	bronchoalveolar lavage fluid
HK	heat-killed
MOI	multiplicity of infection
PTX	pertussis toxin

References

1. Taylor A, Verhagen J, Blaser K, Akdis M, Akdis CA. Mechanisms of immune suppression by interleukin-10 and transforming growth factor-β: the role of T regulatory cells. Immunology. 2006; 117:433–442. [PubMed: 16556256]
2. O'Garra A, Vieira PL, Vieira P, Goldfeld AE. IL-10–producing and naturally occurring $CD4^+$ Tregs: limiting collateral damage. J Clin Invest. 2004; 114:1372–1378. [PubMed: 15545984]
3. Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol. 2001; 19:683–765. [PubMed: 11244051]
4. Groux H, O'Garra A, Bigler M, Rouleau M, Antonenko S, de Vries JE, Roncarolo MG. A $CD4^+$ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. Nature. 1997; 389:737–742. [PubMed: 9338786]
5. Fiorentino DF, Zlotnik A, Vieira P, Mosmann TR, Howard M, Moore KW, O'Garra A. IL-10 acts on the antigen-presenting cell to inhibit cytokine production by Th1 cells. J Immunol. 1991; 146:3444–3451. [PubMed: 1827484]
6. de Waal Malefyt R, Abrams J, Bennett B, Figdor CG, de Vries JE. Interleukin 10(IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. J Exp Med. 1991; 174:1209–1220. [PubMed: 1940799]
7. Alexander WS, Starr R, Fenner JE, Scott CL, Handman E, Sprigg NS, Corbin JE, Cornish AL, Darwiche R, Owczarek CM, et al. SOCS1 is a critical inhibitor of interferon γ signaling and

prevents the potentially fatal neonatal actions of this cytokine. Cell. 1999; 98:597–608. [PubMed: 10490099]

8. Pestka S, Krause CD, Sarkar D, Walter MR, Shi Y, Fisher PB. Interleukin-10 and related cytokines and receptors. Annu Rev Immunol. 2004; 22:929–979. [PubMed: 15032600]
9. Ellis TN, Beaman BL. Interferon-γ activation of polymorphonuclear neutrophil function. Immunology. 2004; 112:2–12. [PubMed: 15096178]
10. Sun K, Salmon SL, Lotz SA, Metzger DW. Interleukin-12 promotes gamma interferon-dependent neutrophil recruitment in the lung and improves protection against respiratory *Streptococcus pneumoniae* infection. Infect Immun. 2007; 75:1196–1202. [PubMed: 17210665]
11. Cooper AM, Adams LB, Dalton DK, Appelberg R, Ehlers S. IFN-γ and NO in mycobacterial disease: new jobs for old hands. Trends Microbiol. 2002; 10:221–226. [PubMed: 11973155]
12. Boss JM. Regulation of transcription of MHC class II genes. Curr Opin Immunol. 1997; 9:107–113. [PubMed: 9039770]
13. Thomas HE, Parker JL, Schreiber RD, Kay TWH. IFN-γ action on pancreatic beta cells causes class I MHC upregulation but not diabetes. J Clin Invest. 1998; 102:1249–1257. [PubMed: 9739059]
14. Magram J, Connaughton SE, Warrier RR, Carvajal DM, Wu CY, Ferrante J, Stewart C, Sarmiento U, Faherty DA, Gately MK. IL-12-deficient mice are defective in IFN γ production and type 1 cytokine responses. Immunity. 1996; 4:471–481. [PubMed: 8630732]
15. Jouanguy E, Döffinger R, Dupuis S, Pallier A, Altare F, Casanova JL. IL-12 and IFN-γ in host defense against mycobacteria and salmonella in mice and men. Curr Opin Immunol. 1999; 11:346–351. [PubMed: 10375558]
16. Novelli F, Casanova JL. The role of IL-12, IL-23 and IFN-γ in immunity to viruses. Cytokine Growth Factor Rev. 2004; 15:367–377. [PubMed: 15450252]
17. Hazlett LD. Pathogenic mechanisms of *P. aeruginosa* keratitis: a review of the role of T cells, Langerhans cells, PMN, and cytokines. DNA Cell Biol. 2002; 21:383–390. [PubMed: 12167240]
18. Breuer K, Wittmann M, Kempe K, Kapp A, Mai U, Dittrich-Breiholz O, Kracht M, Mrabet-Dahbi S, Werfel T. Alpha-toxin is produced by skin colonizing *Staphylococcus aureus* and induces a T helper type 1 response in atopic dermatitis. Clin Exp Allergy. 2005; 35:1088–1095. [PubMed: 16120092]
19. Berg DJ, Davidson N, Kühn R, Müller W, Menon S, Holland G, Thompson-Snipes L, Leach MW, Rennick D. Enterocolitis and colon cancer in interleukin-10-deficient mice are associated with aberrant cytokine production and $CD4^+$ TH1-like responses. J Clin Invest. 1996; 98:1010–1020. [PubMed: 8770874]
20. Singh UP, Singh S, Taub DD, Lillard JW Jr. Inhibition of IFN-γ-inducible protein-10 abrogates colitis in IL-$10^{-/-}$ mice. J Immunol. 2003; 171:1401–1406. [PubMed: 12874231]
21. Yang J, Lindsberg PJ, Hukkanen V, Seljelid R, Gahmberg CG, Meri S. Differential expression of cytokines (IL-2, IFN-γ, IL-10) and adhesion molecules (VCAM-1, LFA-1, CD44) between spleen and lymph nodes associates with remission in chronic relapsing experimental autoimmune encephalomyelitis. Scand J Immunol. 2002; 56:286–293. [PubMed: 12193230]
22. Yoshizawa S, Tateda K, Matsumoto T, Gondaira F, Miyazaki S, Standiford TJ, Yamaguchi K. *Legionella pneumophila* evades gamma interferon-mediated growth suppression through interleukin-10 induction in bone marrow-derived macrophages. Infect Immun. 2005; 73:2709–2717. [PubMed: 15845473]
23. McGuirk P, McCann C, Mills KH. Pathogen-specific T regulatory 1 cells induced in the respiratory tract by a bacterial molecule that stimulates interleukin 10 production by dendritic cells: a novel strategy for evasion of protective T helper type 1 responses by *Bordetella pertussis*. J Exp Med. 2002; 195:221–231. [PubMed: 11805149]
24. Yoshida K, Matsumoto T, Tateda K, Uchida K, Tsujimoto S, Yamaguchi K. Induction of interleukin-10 and down-regulation of cytokine production by *Klebsiella pneumoniae* capsule in mice with pulmonary infection. J Med Microbiol. 2001; 50:456–461. [PubMed: 11339254]
25. Pilione MR, Harvill ET. The *Bordetella bronchiseptica* type III secretion system inhibits gamma interferon production that is required for efficient antibody-mediated bacterial clearance. Infect Immun. 2006; 74:1043–1049. [PubMed: 16428751]

26. Skinner JA, Pilione MR, Shen H, Harvill ET, Yuk MH. *Bordetella* type III secretion modulates dendritic cell migration resulting in immunosuppression and bacterial persistence. J Immunol. 2005; 175:4647–4652. [PubMed: 16177111]
27. Brubaker RR. Interleukin-10 and inhibition of innate immunity to Yersiniae: roles of Yops and LcrV (V antigen). Infect Immun. 2003; 71:3673–3681. [PubMed: 12819047]
28. Zhong G, Fan T, Liu L. Chlamydia inhibits interferon γ-inducible major histocompatibility complex class II expression by degradation of upstream stimulatory factor 1. J Exp Med. 1999; 189:1931–1938. [PubMed: 10377188]
29. de Melker HE, Schellekens JF, Neppelenbroek SE, Mooi FR, Rümke HC, Conyn-van Spaendonck MA. Reemergence of pertussis in the highly vaccinated population of the Netherlands: observations on surveillance data. Emerg Infect Dis. 2000; 6:348–357. [PubMed: 10905967]
30. Skowronski DM, De Serres G, MacDonald D, Wu W, Shaw C, Macnabb J, Champagne S, Patrick DM, Halperin SA. The changing age and seasonal profile of pertussis in Canada. J Infect Dis. 2002; 185:1448–1453. [PubMed: 11992280]
31. von König CH, Halperin S, Riffelmann M, Guiso N. Pertussis of adults and infants. Lancet Infect Dis. 2002; 2:744–750. [PubMed: 12467690]
32. Celentano LP, Massari M, Paramatti D, Salmaso S, Tozzi AE, EUVAC-NET Group. Resurgence of pertussis in Europe. Pediatr Infect Dis J. 2005; 24:761–765. [PubMed: 16148840]
33. Centers for Disease Control and Prevention. Pertussis—United States, 1997–2000. JAMA. 2002; 287:977–979. [PubMed: 11871441]
34. Carbonetti NH, Artamonova GV, Andreasen C, Bushar N. Pertussis toxin and adenylate cyclase toxin provide a one-two punch for establishment of *Bordetella pertussis* infection of the respiratory tract. Infect Immun. 2005; 73:2698–2703. [PubMed: 15845471]
35. Kirimanjeswara GS, Agosto LM, Kennett MJ, Bjornstad ON, Harvill ET. Pertussis toxin inhibits neutrophil recruitment to delay antibody-mediated clearance of *Bordetella pertussis*. J Clin Invest. 2005; 115:3594–3601. [PubMed: 16294220]
36. Wolfe DN, Buboltz AM, Harvill ET. Inefficient Toll-like receptor-4 stimulation enables *Bordetella parapertussis* to avoid host immunity. PLoS One. 2009; 4:e4280. [PubMed: 19169359]
37. Ross PJ, Lavelle EC, Mills KH, Boyd AP. Adenylate cyclase toxin from *Bordetella pertussis* synergizes with lipopolysaccharide to promote innate interleukin-10 production and enhances the induction of Th2 and regulatory T cells. Infect Immun. 2004; 72:1568–1579. [PubMed: 14977963]
38. Mann PB, Wolfe D, Latz E, Golenbock D, Preston A, Harvill ET. Comparative Toll-like receptor 4-mediated innate host defense to *Bordetella* infection. Infect Immun. 2005; 73:8144–8152. [PubMed: 16299309]
39. Parkhill J, Sebaihia M, Preston A, Murphy LD, Thomson N, Harris DE, Holden MTG, Churcher CM, Bentley SD, Mungall KL, et al. Comparative analysis of the genome sequences of *Bordetella pertussis, Bordetella parapertussis* and *Bordetella bronchiseptica*. Nat Genet. 2003; 35:32–40. [PubMed: 12910271]
40. Wolfe DN, Kirimanjeswara GS, Harvill ET. Clearance of *Bordetella parapertussis* from the lower respiratory tract requires humoral and cellular immunity. Infect Immun. 2005; 73:6508–6513. [PubMed: 16177324]
41. Heininger U, Cotter PA, Fescemyer HW, Martinez de Tejada G, Yuk MH, Miller JF, Harvill ET. Comparative phenotypic analysis of the *Bordetella parapertussis* isolate chosen for genomic sequencing. Infect Immun. 2002; 70:3777–3784. [PubMed: 12065521]
42. Mahon BP, Sheahan BJ, Griffin F, Murphy G, Mills KH. Atypical disease after *Bordetella pertussis* respiratory infection of mice with targeted disruptions of interferon-γ receptor or immunoglobulin μ chain genes. J Exp Med. 1997; 186:1843–1851. [PubMed: 9382883]
43. Mahon BP, Mills KH. Interferon-γ mediated immune effector mechanisms against *Bordetella pertussis*. Immunol Lett. 1999; 68:213–217. [PubMed: 10424423]
44. Boehm U, Klamp T, Groot M, Howard JC. Cellular responses to interferon-γ. Annu Rev Immunol. 1997; 15:749–795. [PubMed: 9143706]
45. Schroder K, Hertzog PJ, Ravasi T, Hume DA. Interferon-γ: an overview of signals, mechanisms and functions. J Leukoc Biol. 2004; 75:163–189. [PubMed: 14525967]

46. Conti P, Kempuraj D, Kandere K, Di Gioacchino M, Barbacane RC, Castellani ML, Felaco M, Boucher W, Letourneau R, Theoharides TC. IL-10, an inflammatory/inhibitory cytokine, but not always. Immunol Lett. 2003; 86:123–129. [PubMed: 12644313]
47. Asadullah K, Sterry W, Volk HD. Interleukin-10 therapy—review of a new approach. Pharmacol Rev. 2003; 55:241–269. [PubMed: 12773629]
48. Rigby RJ, Knight SC, Kamm MA, Stagg AJ. Production of interleukin (IL)-10 and IL-12 by murine colonic dendritic cells in response to microbial stimuli. Clin Exp Immunol. 2005; 139:245–256. [PubMed: 15654823]
49. Molski TF, Naccache PH, Marsh ML, Kermode J, Becker EL, Sha'afi RI. Pertussis toxin inhibits the rise in the intracellular concentration of free calcium that is induced by chemotactic factors in rabbit neutrophils: possible role of the "G proteins" in calcium mobilization. Biochem Biophys Res Commun. 1984; 124:644–650. [PubMed: 6093808]
50. Bradford PG, Rubin RP. Pertussis toxin inhibits chemotactic factor-induced phospholipase C stimulation and lysosomal enzyme secretion in rabbit neutrophils. FEBS Lett. 1985; 183:317–320. [PubMed: 2985432]
51. Lautrop H. Epidemics of parapertussis 20 years' observations in Denmark. Lancet. 1971; 297:1195–1198.
52. Cherry JD. The epidemiology of pertussis and pertussis immunization in the United Kingdom and the United States: a comparative study. Curr Probl Pediatr. 1984; 14:7–77.
53. Mahon BP, Brady MT, Mills KH. Protection against *Bordetella pertussis* in mice in the absence of detectable circulating antibody: implications for long-term immunity in children. J Infect Dis. 2000; 181:2087–2091. [PubMed: 10837200]
54. Barnard A, Mahon BP, Watkins J, Redhead K, Mills KH. Th1/Th2 cell dichotomy in acquired immunity to *Bordetella pertussis*: variables in the in vivo priming and in vitro cytokine detection techniques affect the classification of T-cell subsets as Th1, Th2 or Th0. Immunology. 1996; 87:372–380. [PubMed: 8778021]
55. Mahon BP, Ryan MS, Griffin F, Mills KH. Interleukin-12 is produced by macrophages in response to live or killed *Bordetella pertussis* and enhances the efficacy of an acellular pertussis vaccine by promoting induction of Th1 cells. Infect Immun. 1996; 64:5295–5301. [PubMed: 8945580]
56. Wolfe DN, Goebel EM, Bjornstad ON, Restif O, Harvill ET. The O antigen enables *Bordetella parapertussis* to avoid *Bordetella pertussis*-induced immunity. Infect Immun. 2007; 75:4972–4979. [PubMed: 17698566]
57. Restif O, Wolfe DN, Goebel EM, Bjornstad ON, Harvill ET. Of mice and men: asymmetric interactions between *Bordetella* pathogen species. Parasitology. 2008; 135:1517–1529. [PubMed: 18261255]
58. Goebel EM, Wolfe DN, Elder K, Stibitz S, Harvill ET. O antigen protects *Bordetella parapertussis* from complement. Infect Immun. 2008; 76:1774–1780. [PubMed: 18285500]

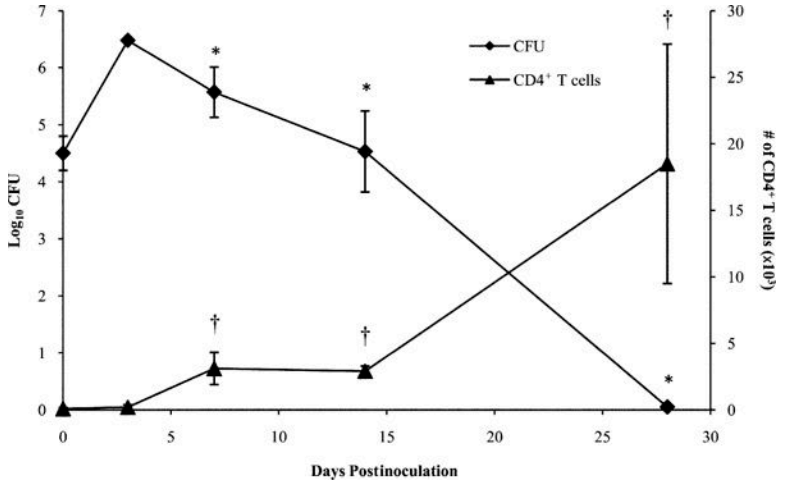

FIGURE 1.

B. parapertussis and CD4$^+$ T cell numbers in murine lungs over time. C57BL/6 mice were inoculated with *B. parapertussis* and sacrificed on day 0, 3, 7, 14, or 28 postinoculation to quantify bacterial numbers in the lungs or CD4$^+$ T cells in the BALF. Bacterial numbers are expressed as the $\log_{10}$ mean CFU of each group of four mice ± SD. CD4$^+$ T cell numbers are expressed as the mean number of cells per lung ± SD. *$p < 0.05$ when comparing bacterial numbers to the peak observed on day 3 postinoculation; †$p < 0.05$ when comparing CD4$^+$ T cell numbers to those of naive mice.

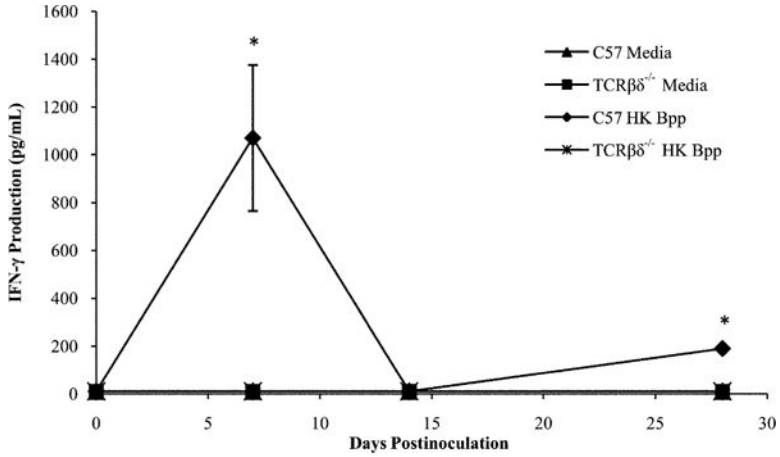

FIGURE 2.

Production of IFN-γ in response to *B. parapertussis*. C57BL/6 and TCRβδ$^{-/-}$ mice were inoculated with *B. parapertussis* and sacrificed 0, 7, 14, or 28 d later. Splenocytes were exposed to media or HK *B. parapertussis* and monitored for the production of IFN-γ. IFN-γ levels are expressed as the mean pictograms per milliliter produced by each group of mice ± SD. *$p < 0.05$ when compared with media-stimulated controls.

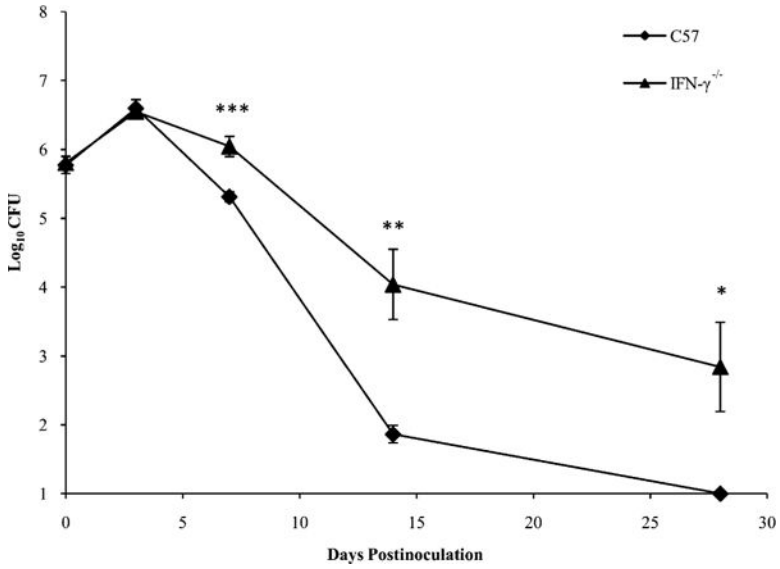

FIGURE 3.

Effect of IFN-γ on clearance of *B. parapertussis*. C57BL/6 and IFN-$\gamma^{-/-}$ mice were inoculated with *B. parapertussis* and sacrificed on day 0, 3, 7, 14, or 28 for the quantification of bacterial numbers in the lungs. CFU are expressed as the $\log_{10}$ mean of each group of mice ± SD. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

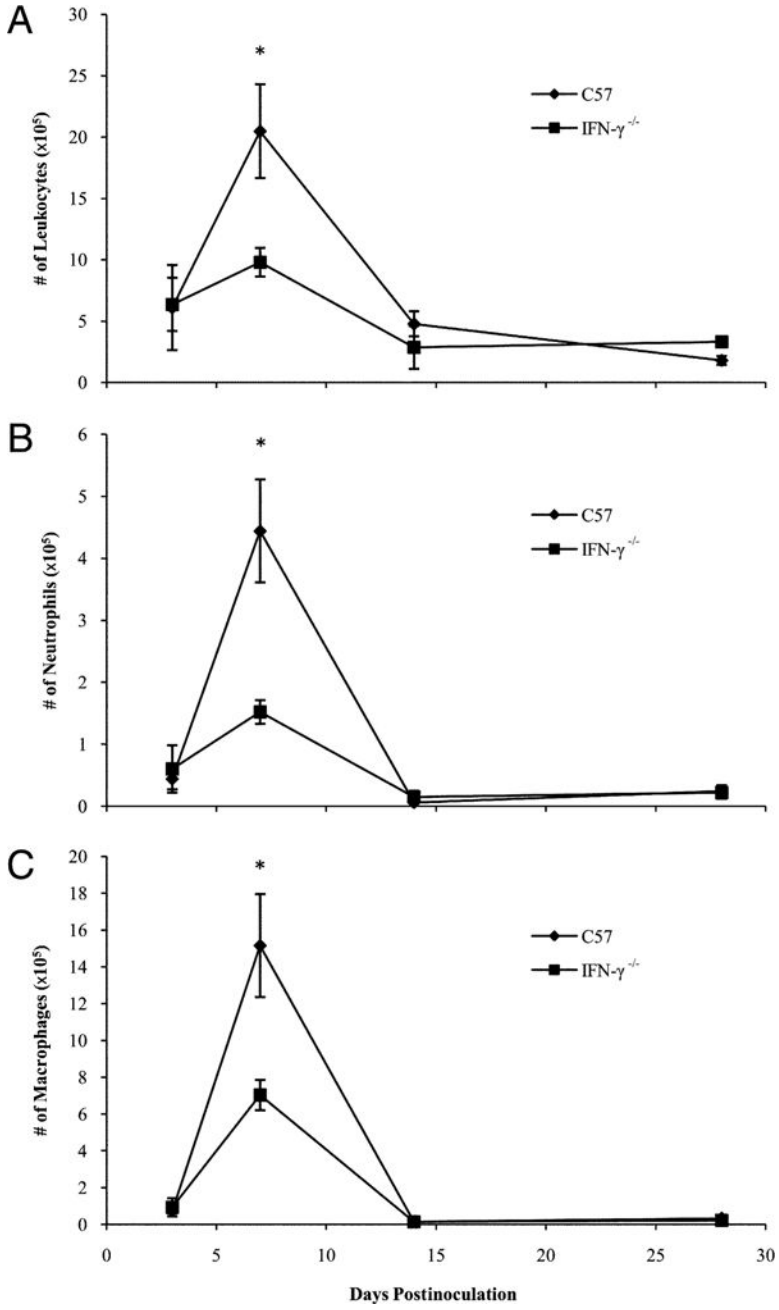

FIGURE 4.

Leukocyte accumulation in response to *B. parapertussis* infection of wild-type and IFN-γ–deficient mice. C57BL/6 and IFN-$\gamma^{-/-}$ mice were inoculated with *B. parapertussis* and sacrificed on day 3, 7, 14, or 28 to quantify (*A*) total leukocytes, (*B*) neutrophils, or (*C*) macrophages in the BALF. WBC numbers are expressed as the mean number of cells per lung ± SD. *$p < 0.05$ when comparing wild-type versus IFN-$\gamma^{-/-}$ mice.

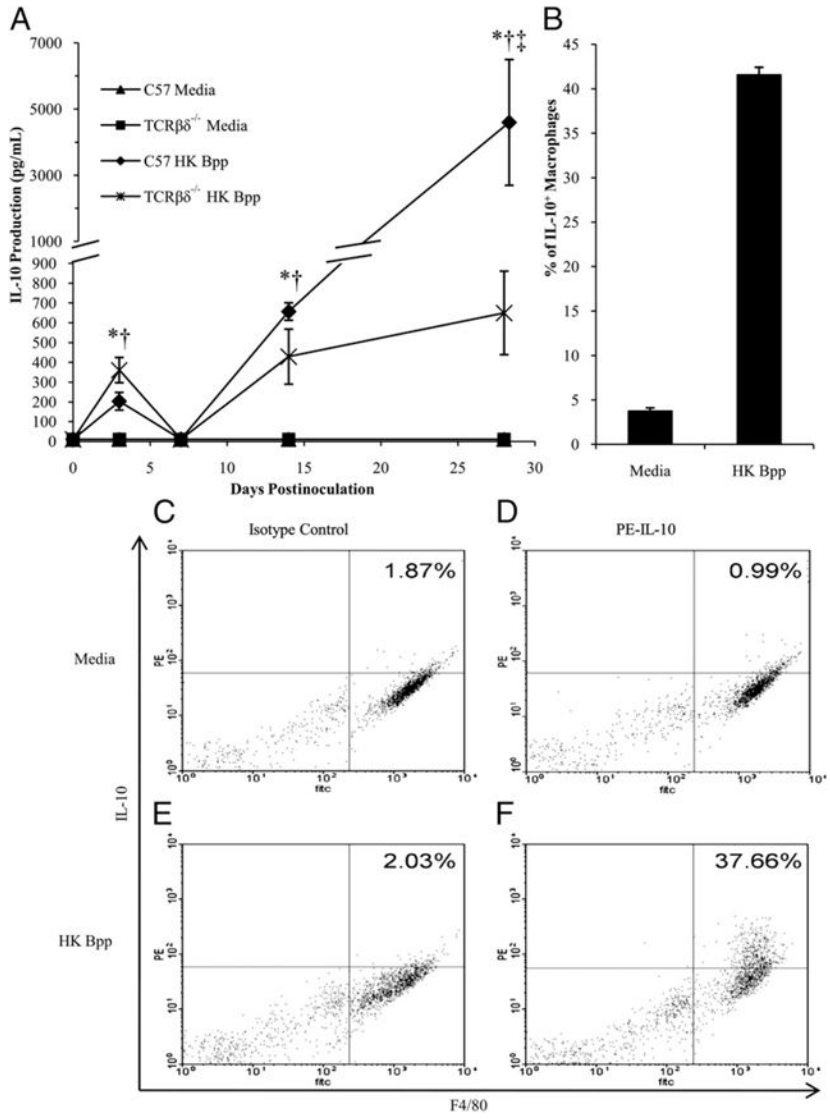

FIGURE 5.

IL-10 production in response to *B. parapertussis*. A, C57BL/6 and TCRβδ$^{-/-}$ mice were inoculated with *B. parapertussis* and sacrificed on day 0, 3, 7, 14, or 28 postinoculation for the quantification of IL-10 production by splenocytes. IL-10 levels are expressed as the mean picograms per milliliter of each group of mice ± SD. $p < 0.05$ when comparing media control to HK *B. parapertussis* stimulated are denoted by asterisks for C57BL/6 or dagger for TCRβδ$^{-/-}$, and double dagger represents $p < 0.05$ when comparing HK *B. parapertussis* stimulated C57BL/6 and TCRβδ$^{-/-}$ mice. The lower limit of detection was 50 pg. *B–F*, Peritoneal macrophages from C57BL/6 mice were stimulated with media (*C, D*) or HK *B. parapertussis* (*E, F*) at an MOI of 5, and the percentages of IL-10-positive macrophages were quantified (*B*). Peritoneal exudate cells were stained with a PE-labeled isotype control Ab (*C, E*) or PE-labeled anti–IL-10 (*D, F*).

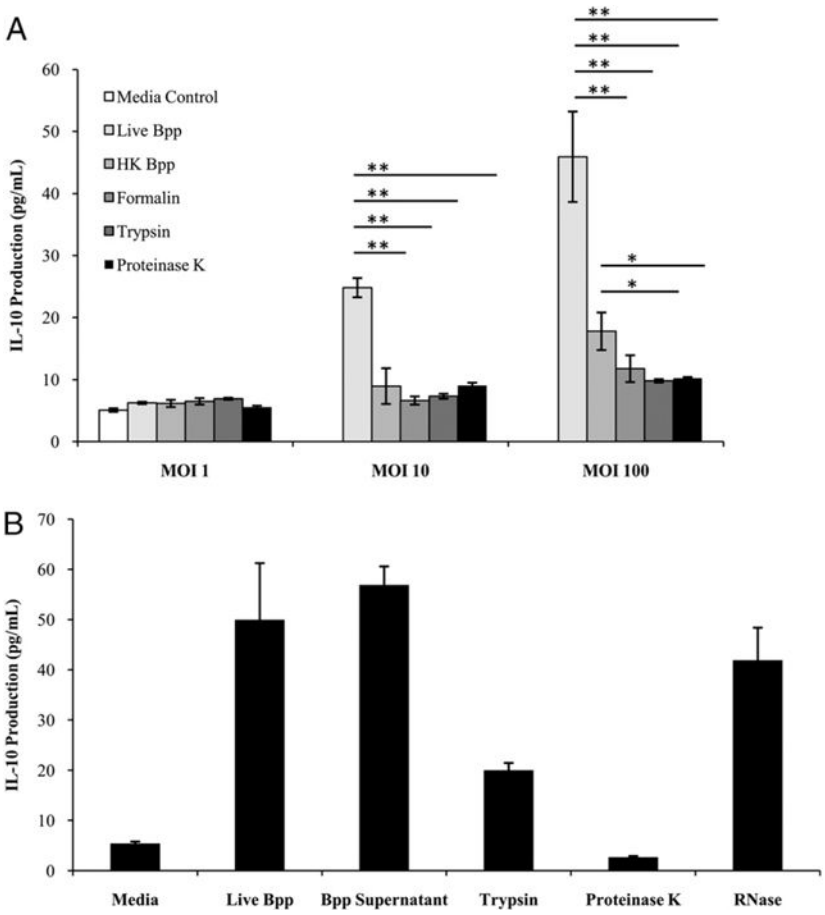

FIGURE 6.

IL-10 production by macrophages in response to live versus HK *B. parapertussis*. A, MH-S cells were stimulated with live *B. parapertussis*, HK *B. parapertussis*, or live *B. parapertussis* treated with heat, formalin, trypsin, or proteinase K at varying MOIs. IL-10 levels are expressed as the mean picograms per milliliter ± SD. *$p < 0.05$; **$p < 0.01$. *B*, MH-S cells were stimulated with live *B. parapertussis*, bacterial culture supernatants, and supernatants that were treated with proteinase K, trypsin, or RNase. Cells were stimulated with these groups for 24 h before supernatants were collected and analyzed for IL-10 quantification via ELISA. IL-10 levels are expressed as the mean picograms per milliliter ± SD.

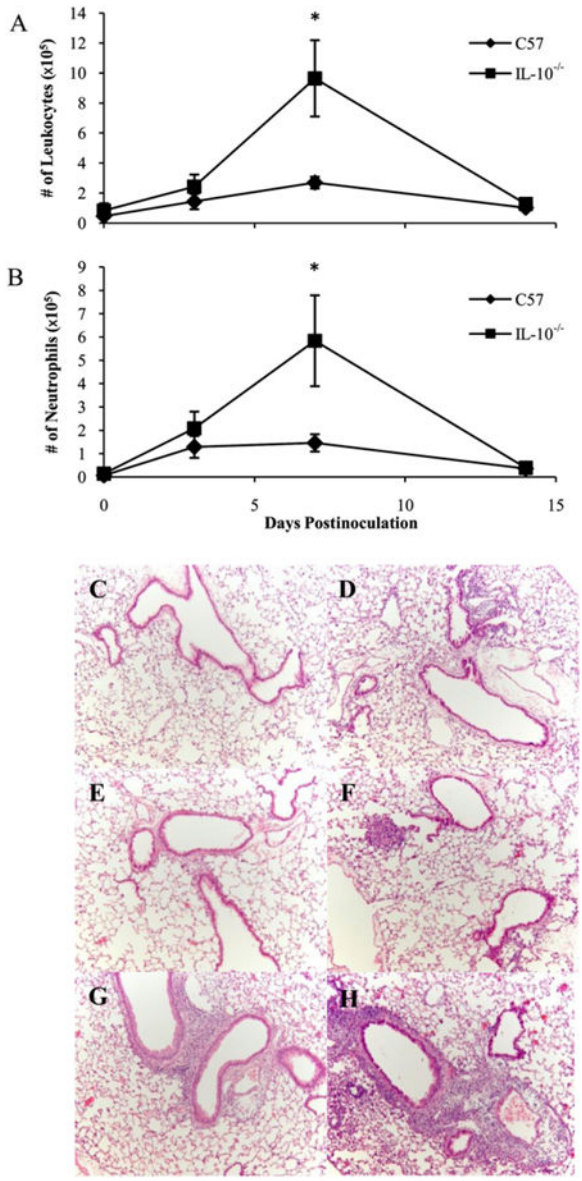

FIGURE 7.

Effect of IL-10 on the inflammatory response to *B. parapertussis* infection. C57BL/6 and IL-10$^{-/-}$ mice were inoculated with *B. parapertussis* and sacrificed on day 0, 3, 7, or 14 to quantify leukocytes (*A*) and neutrophils (*B*) in the BALF. Cell numbers are expressed as the mean number of cells per lung ± SD; *$p < 0.05$. *C–H*, Mice were also sacrificed on day 0 (*C, D*), 3 (*E, F*), or 7 (*G, H*) to evaluate pathology of the lungs of C57BL/6 (*C, E, G*) and IL-10$^{-/-}$ (*D, F, H*) mice. *C–H*, Sections stained with H&E are shown at ×100 magnification.

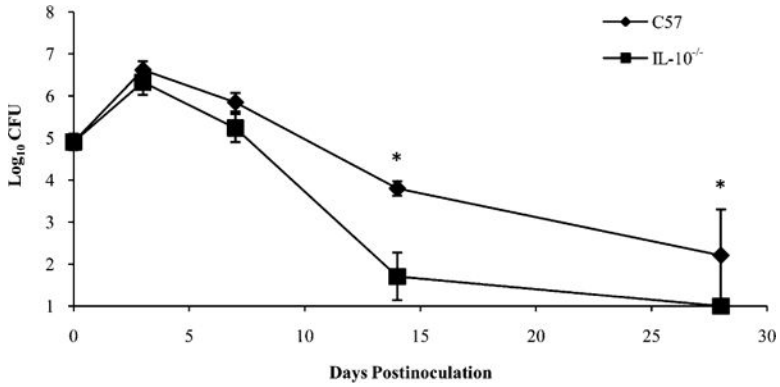

FIGURE 8.

Effect of IL-10 on the clearance of *B. parapertussis* from the lungs. C57BL/6 and IL-$10^{-/-}$ mice were inoculated with *B. parapertussis* and sacrificed on day 0, 3, 7, 14, or 28 to quantify bacterial numbers in the lungs. CFU are expressed as the $\log_{10}$ mean of each group of mice ± SD; $*p < 0.05$.

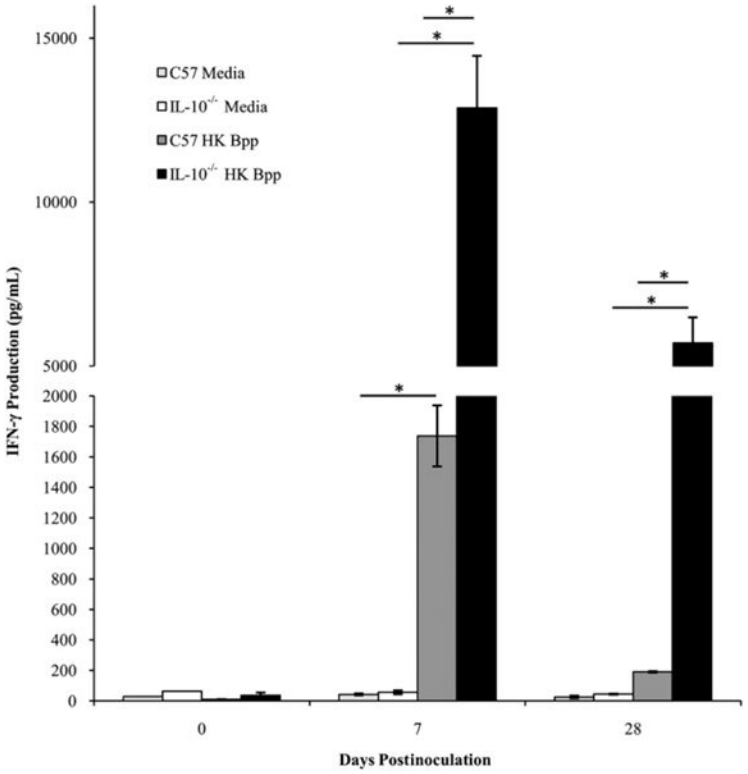

FIGURE 9.

Effect of IL-10 on the IFN-γ response to *B. parapertussis*. C57BL/6 and IL-$10^{-/-}$ mice were inoculated with *B. parapertussis* and sacrificed on day 0, 7, or 28. Splenocytes were exposed to media or HK *B. parapertussis* and monitored for the production of IFN-γ. IFN-γ production was quantified and expressed as the mean picograms per milliliter of each group of mice ± SD. The lower limit of detection was 50 pg; $*p < 0.05$.