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Bordetella Type III Secretion Modulates Dendritic Cell Migration Resulting in Immunosuppression and Bacterial Persistence[1]

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Chronic bacterial infection reflects a balance between the host immune response and bacterial factors that promote colonization and immune evasion. *Bordetella bronchiseptica* uses a type III secretion system (TTSS) to persist in the lower respiratory tract of mice. We hypothesize that colonization is facilitated by bacteria-driven modulation of dendritic cells (DCs), which leads to an immunosuppressive adaptive host response. Migration of DCs to the draining lymph nodes of the respiratory tract was significantly increased in mice infected with wild-type *B. bronchiseptica* compared with mice infected with TTSS mutant bacteria. Reduced colonization by TTSS-deficient bacteria was evident by 7 days after infection, whereas colonization by wild-type bacteria remained high. This decrease in colonization correlated with peak IFN-γ production by restimulated splenocytes from infected animals. Wild-type bacteria also elicited peak IFN-γ production on day 7, but the quantity was significantly lower than that elicited by TTSS mutant bacteria. Additionally, wild-type bacteria elicited higher levels of the immunosuppressive cytokine IL-10 compared with the TTSS mutant bacteria. *B. bronchiseptica* colonization in IL-10$^{-/-}$ mice was significantly reduced compared with infections in wild-type mice. These findings suggest that *B. bronchiseptica* use the TTSS to rapidly drive respiratory DCs to secondary lymphoid tissues where these APCs stimulate an immunosuppressive response characterized by increased IL-10 and decreased IFN-γ production that favors bacterial persistence. *The Journal of Immunology,* 2005, 175: 4647–4652.

Long-term pathogen colonization requires a balance between protective immunity and pathogen-encoded factors that enable the suppression of host immune response. *Bordetella bronchiseptica* uses a type III secretion system (TTSS)[3] to achieve persistent colonization of the murine respiratory tract (1). Colonization occurs despite the apparent development of a humoral IgG2A and Th1-type cellular immune response (2). These findings suggest that the observed host immune response is not sufficient for bacterial clearance. Many chronic diseases, including hepatitis C infection (3, 4), tuberculosis (5), and leishmaniasis (6), occur because an appropriate immune response required for pathogen clearance is not established or is actively suppressed. These pathogens hijack normal host protective immune responses to subvert immune clearance. Deregulation or premature termination of a clearing immune response can lead to long-term pathogen persistence. Chronic pathogens commonly up-regulate the expression of immunosuppressive cytokines such as IL-10 (3, 5–8) and TGF-β (8–10) to subvert clearing immunity. Some viruses capable of establishing chronic infections produce viral homologues to IL-10 (11–16). Although no bacteria-encoded IL-10 homologues have been described, bacteria can induce the secretion of endogenous IL-10 and/or TGF-β from innate immune cells that respond to infection (8, 17–19). McGuirk et al. (8, 20) showed that dendritic cells (DCs) exposed to filamentous hemagglutinin (FHA) from *B. pertussis* secrete IL-10 and inhibit LPS-induced inflammatory cytokine production. Furthermore, McGuirk et al. (8, 20) demonstrated the ability of these IL-10-secreting DCs to induce the clonal expansion of immunosuppressive T regulatory 1 cells capable of suppressing the *Bordetella*-specific Th1 immune response.

DCs are professional APCs that sample the mucosal surface for the presence of foreign Ags. Upon contact and phagocytosis of foreign Ag, DCs mature and migrate to local lymph nodes, where they direct the generation of an adaptive immune response aimed at clearing the Ag (21). The nature and magnitude of the response are dependent on the migratory and maturation states of the DCs (22). Pathogens can suppress the immune response by inhibiting the maturation and migration of DCs from the site of infection to the local lymph nodes (20, 23, 24).

We previously showed that the *B. bronchiseptica* TTSS and type 1 secreted adenylate cyclase toxin (ACT) synergize to alter the maturation state of bone marrow-derived DCs (BMDCs) in vitro (25). The TTSS was shown to up-regulate the surface expression of MHC class II and B.7 costimulatory molecules, whereas ACT down-regulated the surface expression of CD40 and the production of the T cell-polarizing cytokine IL-12. This semimature phenotype is identical with that proposed to direct the generation of regulatory T cells capable of immune suppression (23). *B. pertussis* and *B. bronchiseptica* are highly related subspecies based on genomic comparison (26). Despite this close relationship, *B. pertussis* and *B. bronchiseptica* differ in host range and duration of colonization. *B. pertussis* is a strict human pathogen capable of causing acute respiratory disease. In contrast, *B. bronchiseptica* exhibits a broad host range and normally causes asymptomatic

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[3] Abbreviations used in this paper: TTSS, type III secretion system; ACT, adenylate cyclase toxin; BMDC, bone marrow-derived DC; DC, dendritic cell; FHA, filamentous hemagglutinin.

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persistent respiratory infection in nonhuman mammals (27). Because mice are a natural host for *B. bronchiseptica,* the murine model of infection is ideal for studying host pathogen interactions that lead to bacterial persistence. Previous studies have demonstrated that the *B. bronchiseptica* TTSS is required for long-term colonization of the mouse trachea (1, 28). We hypothesize that *B. bronchiseptica* use the TTSS to interact with APCs at mucosal surfaces to alter the ability of these cells to direct a clearing immune response.

In this study we further characterized the effects of *B. bronchiseptica* type III secretion on DC function and the subsequent modulation of adaptive immune responses in vivo. Because the inhibition of DC maturation/migration is a common strategy used by pathogens to limit the host immune response, we first determined the affect of *B. bronchiseptica* TTSS on in vivo DC migration. Our results show that the *B. bronchiseptica* TTSS actually stimulates rapid DC migration from the respiratory tract to local lymph nodes. Next, we assayed the kinetics of bacterial colonization and the generation of immune responses in animals infected by wild-type or TTSS-defective bacteria. Reduced colonization of the lower respiratory tract by TTSS mutant bacteria correlated temporally with a peak in IFN-γ production by restimulated splenocytes of infected animals. This peak in IFN-γ production was significantly decreased in animals infected with wild-type bacteria, and the wild-type bacteria remained at high colonization levels. After determining the temporal peak of the immune response, we further characterized the cytokine profile present at this time point. Restimulated splenocytes from wild-type infected mice produced more IL-10 than those from animals infected with TTSS mutant bacteria.

Because IL-10-dependent mechanisms are known to mediate microbial colonization, we addressed the role of IL-10 in wild-type *B. bronchiseptica* colonization. In the infection of hosts that do not express the immunosuppressive cytokine IL-10 (IL-$10^{-/-}$ mice), *B. bronchiseptica* exhibited decreased colonization of the murine respiratory tract compared with infection in normal mice, suggesting an important role for IL-10 in the *B. bronchiseptica* colonization strategy. Furthermore, when injected into IL-$10^{-/-}$ mice, wild-type *B. bronchiseptica* exhibited similar levels of colonization as the TTSS mutant bacteria. These findings suggest that *B. bronchiseptica* establishes persistent colonization of the lower respiratory tract by altering the balance between immunostimulatory and immunosuppressive cytokine signals. The *B. bronchiseptica* TTSS plays an important role in this immunomodulatory event by altering DC migration from the respiratory tract to secondary lymphoid tissues, where an immunosuppressive response is generated.

Materials and Methods

Bacteria and mice

B. bronchiseptica strains RB50 (wild-type) and WD3 (ΔbscN) were previously described (28, 29). Before each experiment, a single colony was inoculated in Stainer-Scholte liquid medium and cultured overnight at 37°C with aeration. Before infection, bacteria were subcultured 1/20 into fresh Stainer-Scholte medium and grown at 37°C for 2.5–3 h. These subcultures were diluted with fresh Stainer-Scholte medium to a final concentration of 10^6 bacteria/ml. C57BL/6 mice were then anesthetized by halothane inhalation and intranasally infected with 40 μl of the diluted subcultures (4×10^5 bacteria/mouse). Heat-killed bacteria were obtained by incubation at 65°C for 30 min. C57BL/6 mice, 6–10 wk of age, were obtained from the National Cancer Institute. For IL-$10^{-/-}$ colonization studies, 6- to 10-wk-old B6.129P2-*IL10*^{tm1Cgn}/J (no. 002251) and control C57BL/6J (no. 000664) were obtained from The Jackson Laboratory. Mice were housed in insulator cages and cared for in accordance with institutional animal care and use committee-approved protocols at the University of Pennsylvania School of Medicine animal facility.

In vivo respiratory DC migration

The protocol is adapted from that used by Legge et al. (30). Briefly, CFSE (Molecular Probes) diluted in Iscove's medium to 8 mM was intranasally administered to each mouse (50 μl/mouse) after anesthesia with halothane. Six hours later, mice in groups of three were infected intranasally with 10^5 bacteria in 30 μl. Uninfected mice were instilled with fresh Iscove's medium. After 14 h, cervical lymph nodes were pooled for each group and processed on a wire mesh screen to yield a single-cell suspension. This single-cell suspension was stained with PE-labeled CD86 clone (GL1) and allophycocyanin-labeled CD11c clone (HL3) Ab for 30 min at 4°C in the presence of unlabeled FcγIII/II clone (2.4G2) Ab to block FcR binding. Stained cells were analyzed by flow cytometry using a FACSCalibur (BD Biosciences) and Flow-Jo, version 4.0 (TreeStar).

Restimulation and cytokine determination

At the indicated time points after infection, the spleen from each mouse was independently processed into a single-cell suspension by maceration on a wire mesh screen. Erythrocytes were lysed by treatment with .83% NH_4Cl. Remaining splenocytes were washed and aliquoted in triplicate into 96-well plates. Splenocytes (10^6) were cultured in rich medium for 3 days with and without restimulation by heat-killed wild-type *B. bronchiseptica.* Restimulated wells were treated with 10^7 heat-killed bacteria, whereas unstimulated wells received medium alone. After 3-day restimulation, IFN-γ, IL-10, TNF-α, IL-4, and TGF-β concentrations were determined by ELISA. Each ELISA was preformed on the supernatants of both unstimulated and restimulated splenocytes from the experimental mice. Each cytokine was assayed using a DuoSet ELISA development kit (R&D Systems). The dynamic range between 0 and 2000 pg/ml and specificity have been verified independently and by the manufacturer. Due to variation in the preparation of each standard curve, the limit of detection of each ELISA is ~10 pg/ml. Supernatants from restimulated and unstimulated splenocytes from each mouse were assayed in triplicate and diluted to conform to the dynamic range of each kit. Absorbance of each well was determined by a Synergy HT microplate reader (Bio-Tek) and analyzed by KC4 microplate data analysis software (Bio-Tek). Cytokine production represents the production of cytokine by splenocytes restimulated with heat-killed bacteria normalized to the cytokine production of each splenocyte preparation stimulated by medium alone for each mouse. Unstimulated cytokine production from infected mice was at or below uninfected restimulated levels in all cases.

Results

B. bronchiseptica *TTSS drives respiratory DC migration in vivo*

We hypothesized that *B. bronchiseptica* may use the TTSS to mediate bacterial persistence by affecting DC migration from the site of infection to secondary lymphoid tissues. By modulating DC migration, the generation of adaptive immune responses to *B. bronchiseptica* would be affected. To investigate this possibility, we used a protocol developed by Legge et al. (30) to assess the migration of DCs from the respiratory tract to the draining lymph nodes. Respiratory tracts of C57BL/6 mice were first stained with CFSE, followed by intranasal infection with bacteria. Cervical lymph nodes were then assayed for DC migration by the quantization of fluorescent DCs. Analysis by flow cytometry revealed a 2.8-fold increase in CD11c/CD86/CFSE triple-positive DC migration 14 h after infection with wild-type *B. bronchiseptica* compared with a mutant (Δ*bscN*) defective in type III secretion (Fig. 1). Increased DC migration mediated by wild-type *B. bronchiseptica* was noted by 5 h after infection (data not shown), in agreement with previous reports of DC migration to the mediastinal lymph nodes after wild-type *B. bronchiseptica* infection (31). The type III secretion mutant stimulated only a marginal increase in migrating respiratory DCs compared with uninfected controls, indicating that in the absence of type III secretion, there is little DC migration within the time points studied. By 20 h after infection, basal DC migration and accumulation in the cervical lymph node became indistinguishable from the CFSE-labeled DC accumulation observed during wild-type infection (data not shown). This is due to the rapid basal turnover and migration of these DCs from the respiratory tract and is consistent with the

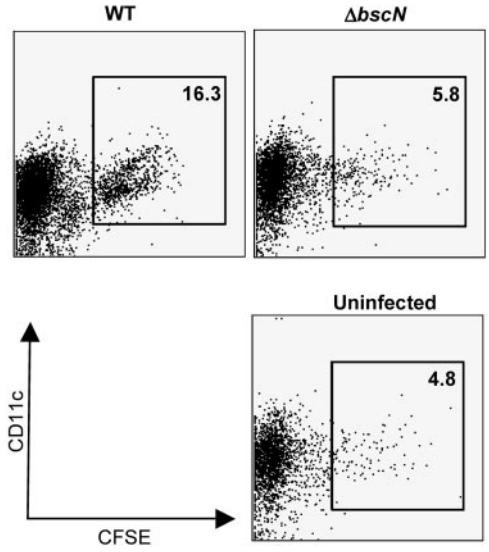

FIGURE 1. *Bordetella* type III secretion is required for increased migration of respiratory DCs to the cervical lymph nodes in infected mice. Respiratory tracts of C57BL/6 mice were stained with CFSE by intranasal instillation (three mice per group). Six hours later, mice were infected with 10^5 wild-type (WT) or type III secretion mutant bacteria. Uninfected mice were instilled with medium alone. After 14 h, cervical lymph nodes were excised, pooled, and processed to determine the extent of respiratory DC migration. All plots are gated on CD86-positive populations. Plots are representative of three independent experiments.

original studies that described this protocol (30). These results demonstrate that the *B. bronchiseptica* TTSS actually does not inhibit DC migration to achieve immunosuppression. On the contrary, wild-type *B. bronchiseptica* induces significant DC migration to respiratory lymph nodes by a TTSS-dependent mechanism.

Bordetella *type III secretion reduces IFN-γ production during the peak immune response and correlates with bacterial persistence*

Because the *Bordetella* TTSS increased the migration of respiratory DCs from the respiratory tract to the draining lymph nodes, we hypothesized that there may be a temporal difference in the generation of the adaptive immune response in mice infected with wild-type bacteria compared with those infected with TTSS-defective bacteria. Such a temporal difference might explain why bacteria defective in type III secretion are more rapidly cleared from the lower respiratory tract. The previous studies of *B. bronchiseptica* TTSS-mediated persistence yielded low resolution colonization data that did not precisely reveal when TTSS mutant bacteria begin to be cleared from the lower respiratory tract (1). Therefore, we repeated these experiments with 2-day points and monitored the generation of the adaptive immune response as measured by IFN-γ production from splenocytes restimulated by heat-killed wild-type *B. bronchiseptica*. As expected, bacteria deficient in type III secretion were cleared form the lung (Fig. 2*A*) and trachea (Fig. 2*B*), but not the nasal septum (Fig. 2*C*), of intranasally infected mice. Increased clearance of the TTSS mutant from the lung and trachea was apparent by day 7 after infection, whereas the colonization levels of wild-type bacteria remained high. Both wild-type and TTSS mutant bacteria elicited peak IFN-γ production levels at 7 days after infection (Fig. 2*D*). However, the amount of IFN-γ produced from restimulated splenocytes from wild-type infected mice was only half that produced by restimulated splenocytes from mice infected with the TTSS mutant. Therefore, there does not appear to be a temporal difference in generation of host responses measured by IFN-γ production in restimulated splenocytes from animals infected with either strain of bacteria. However, there was a significantly higher level of IFN-γ produced from restimulated splenocytes of animals infected with the TTSS mutant bacteria. Furthermore, the temporal peak of IFN-γ production corresponded to the increased clearance of the mutant bacteria from the lower respiratory tract.

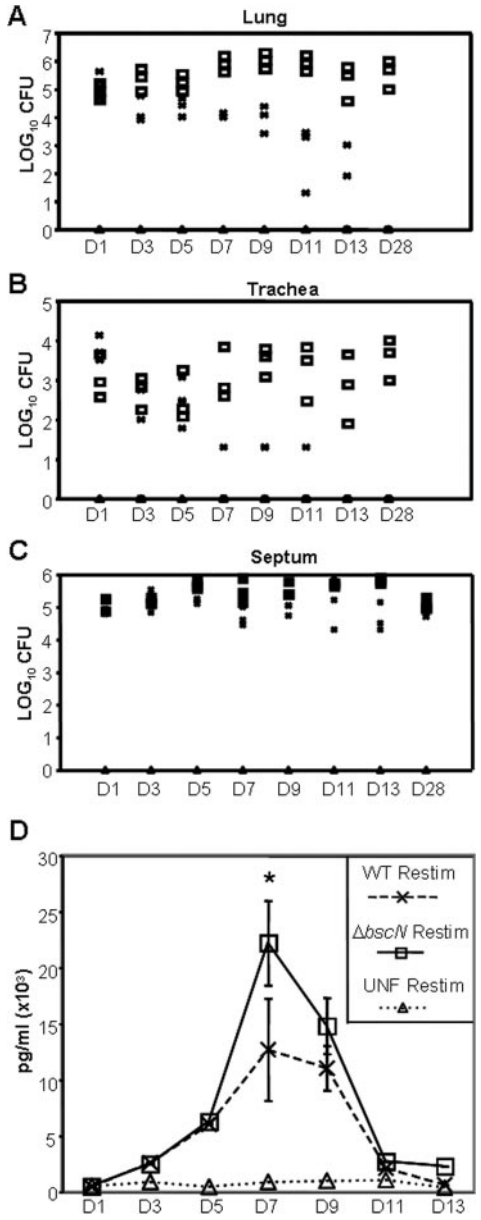

FIGURE 2. Clearance of type III secretion-defective *B. bronchiseptica* from the host corresponds to an increase in IFN-γ production in restimulated splenocytes. C57BL/6 mice were infected with 10^5 wild-type (WT; □) or TTSS mutant (X) *B. bronchiseptica* or were mock infected (△). Three mice were infected for each treatment at each time point. On the indicated days (D) after infection, lungs, trachea, and septum were harvested, and bacterial colonization was assessed (*A–C*). The dashed line represents the limit of detection. Splenocytes from each mouse were restimulated (Restim) with heat-killed *B. bronchiseptica* and assayed for IFN-γ production (*D*). UNF, uninfected. Bars represent the SD of each group. Unpaired *t* test was used to evaluate differences between groups (*, $p < 0.05$).

B. bronchiseptica *type III secretion mediates decreased IFN-γ and increased IL-10 production*

Next, we assayed both unstimulated and restimulated splenocytes from wild-type bacteria-infected and TTSS mutant bacteria-infected mice for the production of immunostimulatory Th1 (IFN-γ) and Th2 (IL-4) cytokines at the peak of the cellular immune response 7 days after infection. Additionally, we assayed for the production of the common immunostimulatory molecule, TNF-α, and immunosuppressive cytokines, IL-10 and TGF-β. As expected, restimulated splenocytes from mice infected with wild-type *B. bronchiseptica* produced decreased IFN-γ (Fig. 3*A*). Conversely, restimulated splenocytes from wild-type bacteria-infected mice produced increased IL-10 (Fig. 3*B*) compared with restimulated splenocytes from mice infected with TTSS mutant bacteria. TNF-α production was not significantly different in restimulated splenocytes from animals infected with either strain of bacteria (Fig. 3*C*). No IL-4 or TGF-β was found (data not shown) within the detection limits of the ELISA used in this experiment (see *Materials and Methods*). These results suggest that the type III secretion system alters the balance between immunosuppressive (IL-10) and immunostimulatory (IFN-γ) cytokine signals. Infection by wild-type bacteria led to higher quantities of IL-10 and lower quantities of IFN-γ from restimulated splenocytes and therefore appears to drive the host system toward an immunosuppressive response.

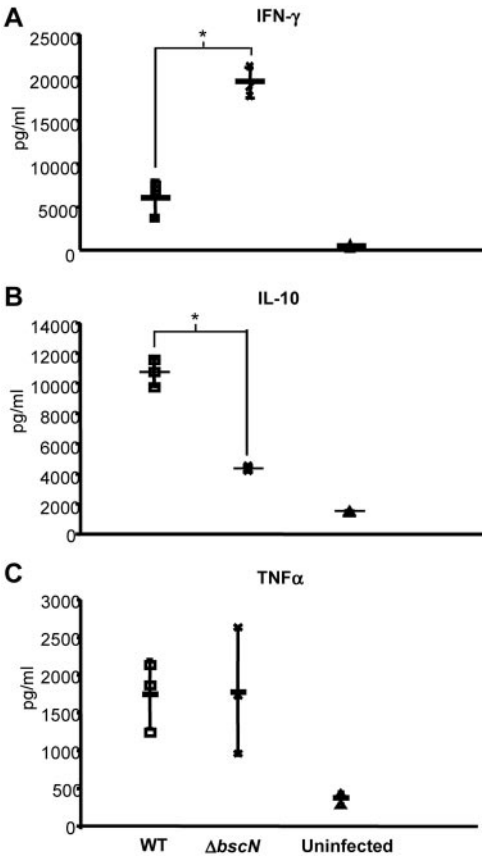

FIGURE 3. *Bordetella* type III secretion mediates decreased IFN-γ and increased IL-10 production in vivo. C57BL/6 mice were infected with wild-type (WT) or TTSS mutant bacteria or were mock infected. On day 7 after infection, splenocytes from each mouse were restimulated with heat-killed *B. bronchiseptica* or were left unstimulated, in triplicate. After 3 days, in vitro culture supernatants were assessed for cytokine content by ELISA. Error bars represent the SD among three independent mice. Horizontal bars indicate the mean values. Unpaired *t* test was used to evaluate differences between groups (∗, $p < 0.05$).

IL-10 is important for complete colonization of the lower respiratory tract by B. bronchiseptica

To determine whether IL-10 has an immunosuppressive role in *B. bronchiseptica* infection, we assessed colonization of wild-type bacteria in IL-10 knockout mice. Wild-type and IL-$10^{-/-}$ knockout mice were intranasally infected with wild-type *B. bronchiseptica*. Respiratory colonization was significantly decreased in the IL-10-deficient background compared with that in wild-type mice (Fig. 4*A*). Bacterial loads in IL-$10^{-/-}$ mice were decreased in both the upper and lower respiratory tract 28 days after infection. We also compared the colonization of wild-type bacteria to TTSS mutant bacteria in IL-$10^{-/-}$ mice. Colonization of wild-type *B. bronchiseptica* was reduced to levels similar to the colonization of TTSS mutant bacteria in IL-$10^{-/-}$ mice (Fig. 4*B*). These results suggest an immunosuppressive role for IL-10 in the colonization strategy of *B. bronchiseptica*. Additionally, these results indicate that in the absence of IL-10 production, the *B. bronchiseptica* TTSS is not able to confer a colonization benefit to the bacteria.

Discussion

For a pathogen to establish a chronic infection, a balance between host immunity and bacterial factors that subvert the host immune response must be reached. In the murine model of *B. bronchiseptica* persistence, the TTSS plays an important role in attaining this balance. Persistent colonization of the lower respiratory tract by *B. bronchiseptica* is dependent on the expression of a functional TTSS. Bacteria defective in type III secretion exhibit accelerated

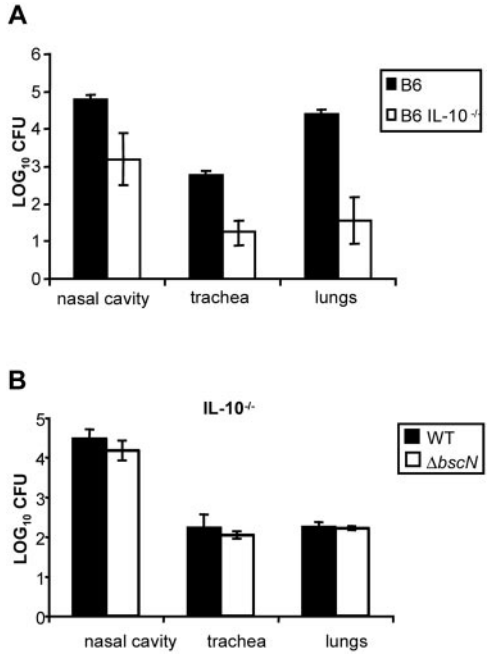

FIGURE 4. IL-10 is required for high level colonization of the lower respiratory tract by *B. bronchiseptica*. Wild-type (WT) C57BL/6J and C57BL/6J IL-$10^{-/-}$ mice were intranasally infected with 10^5 bacteria (three mice per group). *A*, On day 28 after infection, the nasal cavity, trachea, and lungs were harvested and assayed for *B. bronchiseptica* colonization. Error bars represent the SD among the three mice in each group. *B*, C57BL/6J IL-$10^{-/-}$ mice were intranasally infected with 10^5 bacteria (three mice per group). On day 14 after infection, the nasal cavity, trachea, and lungs were harvested and assayed for *B. bronchiseptica* colonization. Error bars represent the SD among the three mice in each group.

clearance from the trachea and higher anti-*Bordetella* Ab titers (1). We previously showed that the *B. bronchiseptica* TTSS and ACT synergize to drive BMDCs into a semimature state. Phenotypically these DCs had high surface expression of MHC class II, CD80, and CD86 (TTSS dependent) and low CD40 surface expression (ACT dependent). IL-12 cytokine production was also reduced in an ACT-dependent manner. We hypothesized that these DCs would function to shift the host immune response away from a clearing Th1-type T cell response characterized by IFN-γ production toward an immunosuppressive response characterized by the production of IL-10 and/or TGF-β. In this study we assessed in vivo DC migration in the context of wild-type and TTSS mutant infections and investigated the balance of immunostimulatory and immunosuppressive cytokines that resulted from these infections.

First, we conducted an in vivo DC migration assay to test the possibility that type III secretion may be down-regulating the *B. bronchiseptica*-specific host immune response by inhibiting DC migration from the respiratory tract to the local lymph nodes. Instead, we found that wild-type *B. bronchiseptica* elicited more respiratory DC migration than TTSS mutant bacteria. The TTSS mutant bacteria-infected mice showed only a marginal increase in DC migration over the basal DC migration seen in uninfected mice. This indicated that the immunosuppressive function of the TTSS is not to inhibit DC migration. Instead, DCs infected by wild-type bacteria may be driving the host toward an immunosuppressive response that permits bacterial persistence.

Despite the difference in DC migration, IFN-γ production from restimulated splenocytes peaked at 7 days after infection for both wild-type and TTSS mutant bacteria-infected mice. However, there was a significant decrease in the amount of IFN-γ produced by splenocytes from wild-type bacteria-infected mice compared with that from splenocytes in TTSS mutant bacteria-infected mice. This peak on day 7 after infection correlated with the beginning of reduced colonization of the lower respiratory tract by the TTSS mutant. A recent report by Widney et al. (32) showed that the production of IFN-γ in the lungs of mice infected with *B. bronchiseptica* peaked 2 days after infection and was not associated with reduced bacterial load. Together with our data, this suggests that that the production of systemic, and not local, IFN-γ is important for bacterial clearance. Further investigation of the cytokines produced by day 7 restimulated splenocytes showed that in addition to decreased IFN-γ, splenocytes from wild-type *B. bronchiseptica*-infected mice secreted more IL-10 than those from TTSS mutant bacteria-infected mice. No IL-4 or TGF-β was detected in the supernatants of these assays. The type III secretion-dependent shift from IFN-γ production to IL-10 production in the absence of IL-4 suggests that T regulatory type 1 responses might be induced in vivo (20, 23) during wild-type *B. bronchiseptica* infection (20). The number of total $CD4^+CD25^+$ populations was higher in the spleens of wild-type bacteria-infected mice (data not shown). However, both T regulatory cells and responding Th1 cells can show a high $CD4^+CD25^+$ phenotype, and we are unable to distinguish between them at this point. We are currently assaying the functionality of these induced cells and their ability to suppress cell-mediated immune responses to foreign Ags.

The importance of IL-10 in persistence of wild-type *B. bronchiseptica* was demonstrated by reduced bacterial colonization in IL-$10^{-/-}$ mice compared with control mice. Decreased colonization in IL-$10^{-/-}$ mice has been reported for many microorganisms (33, 34). These colonization defects may be due to elevated basal IFN-γ production in these mice. For a colonizing microorganism, this underscores the importance of achieving and maintaining an ideal IFN-γ/IL-10 balance in vivo. This balance probably establishes an equilibrium between the microorganism and the host that simultaneously limits excessive growth of the microorganism while also limiting an excessive immune response that would completely clear it from the host. Because wild-type *B. bronchiseptica* and TTSS mutant bacteria colonize the respiratory tracts of IL-$10^{-/-}$ mice at similar levels, we conclude that TTSS-induced IL-10 plays a central role in long-term colonization of the host respiratory tract.

This study suggests that the *B. bronchiseptica* TTSS actively drives DCs to secondary lymphoid tissues, where they function to stimulate immunosuppressive immune responses that contribute to long-term colonization of the murine respiratory tract. If these DCs were fully immunostimulatory and in the classical DC1 phenotype (20), they would generate a vigorous Th1 response capable of rapidly clearing the bacteria from the respiratory tract. However, wild-type bacteria are able to persist in the lower respiratory tract even though they appear to drive rapid DC migration to local lymph nodes. Therefore, these DCs, in turn, most likely drive the host toward an immunosuppressive response, as demonstrated by the high IL-10/low IFN-γ profile we have presented in this study. These observations are consistent with our overall hypothesis that the primarily function of the *Bordetella* TTSS is to down-regulate host immune responses to allow persistent infection by the bacteria. We are currently investigating the source of secreted systemic IL-10 in wild-type infection and focusing on the role of induced regulatory T cells. Additionally, we are determining the mechanism by which the *B. bronchiseptica* TTSS stimulates DCs to migrate from the periphery to the local lymph nodes. Understanding the mechanism of *B. bronchiseptica* TTSS-dependent immune modulation may elucidate novel mechanisms by which persistent Gram-negative bacteria actively tailor the immune response to favor colonization over clearance.

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Disclosures

The authors have no financial conflict of interest.

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