**Bordetella pertussis** Inhibition of Interleukin-12 (IL-12) p70 in Human Monocyte-Derived Dendritic Cells Blocks IL-12 p35 through Adenylate Cyclase Toxin-Dependent Cyclic AMP Induction

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*Bordetella pertussis* is the causative agent of whooping cough, possesses an array of virulence factors, including adenylate cyclase toxin (ACT), relevant in the establishment of infection. Here we better define the impact of cyclic AMP (cAMP) intoxication due to the action of ACT on dendritic cell (DC)-driven immune response, by infecting monocyte-derived DC (MDDC) with an ACT-deficient *B. pertussis* mutant (ACT18HS19) or its parental strain (WT18323). Both strains induced MDDC maturation and antigen-presenting cell functions; however, only ACT18HS19 infected MDDC-induced production of interleukin-12 (IL-12) p70. Gene expression analysis of the IL-12 cytokine family subunits revealed that both strains induced high levels of p40 (protein chain communal to IL-12 p70 and IL-23) as well as p19, a subunit of IL-23. Conversely only ACT18HS19 infection induced consistent transcription of IL-12 p35, a subunit of IL-12 p70. Addition of the cAMP analogous β-butyril-cAMP (β-cAMP) abolished IL-12 p70 production and IL-12 p35 expression in ACT18HS19-infected MDDC. ACT18HS19 infection induced the expression of the transcription factors interferon regulatory factor 1 (IRF-1) and IRF-8 and of beta interferon, involved in IL-12 p35 regulation, and the expression of these genes was inhibited by β-cAMP addition and in WT18323-infected MDDC. The concomitant expression of IL-12 p70 and IL-23 allowed ACT18HS19 to trigger a more pronounced T helper 1 polarization compared to WT18323. The present study suggests that ACT-dependent cAMP induction leads to the inhibition of pathways ultimately leading to IL-12 p35 production, thus representing a mechanism for *B. pertussis* to escape the host immune response.

*Bordetella pertussis* is the causative agent of whooping cough, a respiratory disease representing a severe and life-threatening illness, particularly in infants and children (22, 30). Many aspects of pertussis pathogenesis and protective mechanisms are not fully understood (32). Upon infection, bacteria colonize the mucosa of the upper respiratory tract and synthesize a variety of virulence factors, including adhesins and toxins (28). Adenylate cyclase toxin (ACT) plays an important threefold role in the establishment of infection: (i) it cooperates with other virulence factors in the colonization of the respiratory tract (5, 16, 25), (ii) it recruits and kills inflammatory leukocytes (18), (iii) it inhibits both Fc receptor-mediated attachment and phagocytosis of *B. pertussis* by neutrophils (41).

ACT is a secreted protein composed of two independent domains: the N-terminal 400 amino acids bearing a calmodulin-dependent adenylate cyclase activity and the C-terminal region carrying a calcium-dependent hemolytic activity (6, 42). The C-terminal region binds to the CD11b/CD18 αβ2 integrin, expressed on innate immune cells, including macrophages and dendritic cells (DC) (19). Upon receptor binding, the N-terminal domain is translocated into the cell and activated by intracellular calmodulin (21). The intracellular ACT activation causes a supraphysiologic cyclic AMP (cAMP) production, a process termed intoxication (17). Intoxication leads to inhibition of phagocytosis of immune effector cells and causes an oxidative burst responsible for the induction of macrophage apoptosis, allowing persistence of *B. pertussis* in the host and progression of infection (18).

DC play a central role in the immune system, linking innate and adaptive immunity. In their immature stage, DC act as sentinels able to capture microbial antigens at the site of infection. This encounter drives the maturation process, a complex rearrangement of gene expression that allows cytokine production and DC migration to lymph nodes where they present antigens to naive T cells and polarize the adaptive immune response (4, 33, 36).

We have previously shown that *B. pertussis*, while possessing a low susceptibility to be internalized by—and to survive in—monocyte-derived DC (MDDC), triggers the onset of the maturation program and modulates cytokine production and antigen-presenting cell functions (11). In particular, *B. pertussis*-infected MDDC are unable to induce the production of interleukin-12 (IL-12) p70, a crucial regulatory cytokine in the Th1 polarization of immune response (39), nevertheless driving Th1 immunity, and IL-23 may indirectly contribute to this polarization (11).

IL-12 p70 and IL-23 are members of the type I heterodimeric cytokine superfamily and are composed of two heterologous chains differently regulated: p40 and p35 compose the IL-12 p70 and p19 chains and p40 composes the IL-23 chain (8, 23, 26, 39). These cytokines represent important physiological
regulators in vivo for cellular response to microbial infections and are secreted by DC and phagocytes in response to pathogens (23). In particular, IL-23 has been demonstrated as having a role in resistance to several pathogens, such as gram-negative pulmonary pathogens (20) or Mycobacterium tuberculosis (24). Recent studies have shown IL-23 is a regulatory factor that promotes the expansion of IL-17-producing T cells (Th17) implicated in the inflammation and autoimmune process, which are distinct from Th1 cells, even if the relationship between Th17 and Th1 cells remains unclear (31).

Since ACT significantly inhibits lipopolysaccharide (LPS)-driven IL-12 p70 production in human and murine DC (3, 34), in the present study, we tried to better define the impact of B. pertussis and, in particular, of ACT on human DC-driven T cell immune response to this pathogen. Human MDDC were infected with a B. pertussis ACT-deficient mutant (ACT−/HS19) (25) and its parental strain (WT18323), and their capacity to affect MDDC functions were compared. To complement ACT activity, exogenous α,β-nutril-cAMP (α-β-cAMP), a cAMP-analogue molecule, was used to induce cell intoxication. These approaches allowed us to unravel the crucial role played by ACT-induced intracellular cAMP accumulation in the regulation of IL-12 cytokine family expression and of T helper immune response in the host.

MATERIALS AND METHODS

Reagents. LPS from Escherichia coli, polymyxin B, brefeldin A, phytomitogen-glutamin (PHA), phorbol 12-myristate-13-acetate (PMA), r-α-cAMP, ionomycin, and recombinant Bordetella pertussis ACT (rACT) were obtained from Sigma Chemical Co. (St. Louis, MO). Human recombinant (hr) granulocyte-macrophage colony-stimulating factor and hrIL-4 were obtained from Novartis Pharma AG (Basel, Switzerland). hrIL-2 was obtained from Roche (Basel, Switzerland).

Bacterial strains and growth conditions. B. pertussis strain WT18323 (ATCC reference strain 97-97) and its isogenic ACT−/HS19 mutant (25) were incubated onto charcoal agar plates supplemented with 10% sheep blood (Oxoid, Basingstoke, United Kingdom) and grown at 37°C for 72 h to visualize hemolysis and plated again on charcoal agar for 48 h at 37°C. Bacteria were then collected and resuspended in 5 ml of phosphate-buffered saline. The bacterial concentration was estimated by measuring the optical density at 600 nm, and the suspension was adjusted to a final concentration of 10^7 CFU/ml.

Purification and culture of MDDC. Human monocytes were purified from peripheral blood of healthy donors as described previously (2). CD14+ cells were cultured at 5 × 10^6/ml in RPMI 1640 (ICN-Flow, Aurora, OH) supplemented with heat-inactivated 10% LPS-screened fetal bovine serum (Limulus amebocyte lysate, Sigma) (hereafter defined as complete medium) at 37°C in 5% CO2. Supernatants were collected after 24 h, and IL-10, IL-12 p70, and IL-12 p40 production were assessed by enzyme-linked immunosorbent assay (ELISA) (Quintikine; R&D Systems, Inc., Minneapolis, MN) with sensitivities of 3.9 pg/ml for IL-10, 5 pg/ml for IL-12 p70, and 31.2 pg/ml for IL-12 p40. The optical density was measured with a Bio-Rad (Philadelphia, PA) 3500 UV microplate reader at 450 nm.

To evaluate intracellular cAMP production, MDDC (1 × 10^6) were cultured with B. pertussis strain or rACT for 1 h and 30 min. Cultures were then washed, incubated with 1 M HCl for 20 min, and neutralized with 500 μl of complete medium at 37°C in 5% CO2. cAMP concentration was assessed by ELISA (Quintikine; R&D Systems, Inc., Minneapolis, MN).

Intracellular staining. Intracellular staining was performed in T cells after polarization experiments (12). The staining was performed in the presence of brefeldin A (10 μg/ml). T lymphocytes were stained for cell surface antigens using pretitrated fluorochrome-conjugated anti-CD3 MAb. Cells were then fixed and permeabilized as described by BD Biosciences (Cytofix-Cytoperm and Perm/Wash protocol) and stained with a predetermined optimal concentration of fluorochrome-conjugated anti-CD3 MAb. Cells were then fixed for 20 min at 4°C and washed twice with PBS supplemented with 0.05% NaN3 and 0.02% sodium azide.

mRNA cytokine expression by TaqMan real-time RT-PCR analysis. To measure cytokine mRNA expression, TaqMan real-time reverse transcript PCR (RT-PCR) analysis was used (Applied Biosystems, Foster City, CA). Total RNA was extracted from MDDC at different time points, and reverse transcription was carried out as previously described (1). TaqMan assays were performed according to the manufacturer’s instructions with an ABI 7700 thermocycler (Applied Biosystems) (11). PCR was performed by amplifying the target cDNA (p40, p35, p19, interferon regulatory factor 1 (IRF-1), IRF-8, and interferon β1 (IFN-β1)) template and using the β-actin cDNA as an endogenous control. A probe, labeled at the 5' end with a fluorescent reporter and at the 3' end with a quencher, annealing the amplicon, was added to PCR mix. During PCR the 5' to 3' nuclease activity of TaqMan polymerase cleaves the probe, resulting in displacement of the quencher from the reporter and release of a fluorescent signal.
Specific primers and probes were obtained from Applied Biosystems. Data obtained were analyzed with the PE relative quantification software of Applied Biosystems. Specific mRNA transcript levels were expressed as relative increases compared to basal condition.

Statistical analysis. Statistical descriptive analyses were carried out using the SPSS, Inc. (Chicago, IL) statistical package. Differences between mean values were assessed by two-tailed Student’s t test and were statistically significant for P values of < 0.05.

RESULTS

B. pertussis infection of MDDC leads to intracellular cAMP accumulation. cAMP is a ubiquitous messenger which integrates many processes in the cell and plays a central role in the regulation of the immune response, including functions of DC (3, 13, 14).

In an initial set of experiments, we measured intracellular cAMP levels induced in MDDC upon B. pertussis infection.

![Image of a graph showing intracellular cAMP accumulation in MDDC infected with WT18323 and its isogenic ACT^-18HS19 mutant strain.](http://iai.asm.org/)

**TABLE 1. Intracellular cAMP levels in MDDC infected with WT18323 and ACT^-18HS19 strains**

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>cAMP (pmol/ml)</th>
</tr>
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<tbody>
<tr>
<td>WT18323</td>
<td>876 ± 21.4</td>
</tr>
<tr>
<td>ACT^-18HS19</td>
<td>1.7 ± 0.3</td>
</tr>
<tr>
<td>ACT^-18HS19 + D-cAMP (100 μg/ml)</td>
<td>11.64 ± 79.5</td>
</tr>
<tr>
<td>rACT (1 μg/ml)</td>
<td>834 ± 5.4</td>
</tr>
<tr>
<td>None</td>
<td>0.9 ± 0.6</td>
</tr>
</tbody>
</table>

a iMDDC were treated with indicated stimuli for 1.5 h, and cell lysate was analyzed by a specific ELISA test.

b Values are means ± standard errors of results from three independent experiments.

c P < 0.05 by Student’s t test for comparison between ACT^-18HS19 and WT1832 or rACT.

d P < 0.05 by Student’s t test for comparison between ACT^-18HS19 plus D-cAMP and all other stimuli.

The data obtained, reported in Table 1, showed high levels of intracellular cAMP induced by WT18323, similar to those detected after incubation with rACT, used as positive control. In contrast, when MDDC were infected with ACT^-18HS19, intracellular cAMP levels were almost undetectable, showing that B. pertussis ACT is the principal virulence factor causing intracellular cAMP accumulation. The addition of D-cAMP, a cAMP-analogous molecule, to ACT^-18HS19-treated MDDC restored almost the same level of intracellular cAMP induced by WT18323-treated MDDC (Table 1).

Phenotypic maturation and APC activities of B. pertussis infected MDDC. A first step in the transition of MDDC from phagocytic to antigen-presenting cell (APC) functions after the encounter with a pathogen is the modification of surface phenotype (4, 33, 36). iMDDC were infected with WT18323 or ACT^-18HS19, and phenotypic maturation was assessed. Figure 1A shows a representative histogram analysis, while Table 2 summarizes the surface expression of the maturation marker CD83, the costimulatory molecule CD80, and HLA-

**TABLE 2. MDDC infected with WT18323 and ACT^-18HS19 are phenotypically mature**

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>CD80 (MFI)</th>
<th>CD83 (% positive cells)</th>
<th>HLA-DR (MFI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT18323</td>
<td>33 ± 5 (2.6 ± 0.2)</td>
<td>51 ± 7 (0.04 ± 0.02)</td>
<td>144 ± 20 (2.2 ± 0.3)</td>
</tr>
<tr>
<td>ACT^-18HS19</td>
<td>47 ± 6 (2.5 ± 0.2)</td>
<td>47 ± 5 (0.06 ± 0.03)</td>
<td>150 ± 27 (2.5 ± 0.3)</td>
</tr>
<tr>
<td>LPS</td>
<td>35 ± 4 (2.4 ± 0.3)</td>
<td>52 ± 7 (0.04 ± 0.03)</td>
<td>170 ± 39 (2.4 ± 0.2)</td>
</tr>
<tr>
<td>None</td>
<td>18 ± 3 (2.5 ± 0.3)</td>
<td>8 ± 3 (0.05 ± 0.02)</td>
<td>135 ± 36 (2.3 ± 0.3)</td>
</tr>
</tbody>
</table>

a iMDDC were treated with indicated stimuli for 24 h and analyzed for indicated surface markers associated with mature phenotype. Mean expression ± standard error of results from 14 experiments is indicated.

The data obtained, reported in Table 1, showed high levels of intracellular cAMP induced by WT18323, similar to those detected after incubation with rACT, used as positive control. In contrast, when MDDC were infected with ACT^-18HS19, intracellular cAMP levels were almost undetectable, showing that B. pertussis ACT is the principal virulence factor causing intracellular cAMP accumulation. The addition of D-cAMP, a cAMP-analogous molecule, to ACT^-18HS19-treated MDDC restored almost the same level of intracellular cAMP induced by WT18323-treated MDDC (Table 1).

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DR, all measured 24 h after infection with living bacteria. Both strains induced surface expression of the maturation markers at levels comparable to those induced by E. coli LPS, and statistically significant differences with respect to untreated MDDC were reached for the expression of the CD80 and CD83 maturation markers analyzed (Table 2).

To evaluate a key role played by DC in vivo, such as in APC functions, we performed mixed lymphocyte reaction experiments by coculturing infected MDDC and allogeneic purified T lymphocytes. Figure 1B shows that both WT18323- and ACT^-18HS19-infected MDDC were able to stimulate alloreactive T-cell proliferation at comparable levels with respect to E. coli LPS, while untreated MDDC scarcely induced allogeneic T-cell proliferation.

**B. pertussis affects production of IL-12 p70 through a cAMP-dependent pathway.** IL-12 and IL-10 production is a key step in the regulation of the immune response exerted by DC (2, 9, 12, 39). In a previous study, we showed that WT18323 infection, while promoting high levels of IL-10, does not induce IL-12 p70 secretion in MDDC (11). Here, we compared the release of these two cytokines in response to infection with WT18323 or ACT^-18HS19 strains.

The WT18323 strain was confirmed to be almost unable to induce IL-12 p70 secretion in MDDC (11), while ACT^-18HS19 released statistically significant higher levels of IL-12 p70 (P = 0.01) (Fig. 2A). To assess whether IL-12 p70 inhibition caused by WT18323 infection was dependent on intracellular cAMP induction, we performed infection experiments using ACT^-18HS19 in the presence of t-cAMP. As shown in Fig. 2A, the addition of t-cAMP dramatically inhibited, to almost undetectable levels, IL-12 p70 production.

The WT18323 strain infection induced secretion of high levels of IL-10 in MDDC cultures, confirming previous results (11). ACT activity did not affect IL-10 production by MDDC. Indeed, ACT^-18HS19- and WT18323-infected cells released similar amounts of cytokine, while there was no statistically significant reduction after t-cAMP addition (Fig. 2B).

IL-12 and IL-23 are heterodimeric proteins, composed of p40/p35 and p40/p19 dimers, respectively, involved in Th polarization of immune response (23, 31, 39). Since we had previously demonstrated that infection with strain WT18323 selectively blocks the expression of p35, the smaller subunit of IL-12 p70, while it induces IL-23 (11), we next compared IL-12 and IL-23 subunit gene expression in WT18323- or ACT^-18HS19-infected MDDC, using quantitative RT-PCR.

Both strains induced high levels of p40 and consistent levels of p19 mRNA expression, particularly ACT^-18HS19. The WT18323 strain was confirmed to be unable to express p35, while ACT^-18HS19 induced consistent p35 mRNA transcription (Fig. 3A).

MDDC infection with ACT^-18HS19 in the presence of t-cAMP resulted in reduction of p40 and p19 mRNA expression and the abrogation of p35 mRNA expression (Fig. 3A). These data were confirmed at the protein level for IL-12 p40 and IL-12 p70 (Fig. 3B). Also, in this case, ACT^-18HS19-treated MDDC released statistically significant higher levels of IL-12 p70 and IL-12 p40 than WT18323 or ACT^-18HS19 in the presence of t-cAMP-treated MDDC (Fig. 3B).

Thus, these experiments yielded evidence of a direct role played by bacterial ACT activity in suppression of IL-12 family protein chains and, in particular, in blocking p35 mRNA induction in human MDDC through intracellular cAMP accumulation.

**B. pertussis-mediated cAMP accumulation inhibits IRF-1, IRF-8, and IFN-β mRNA expression in human MDDC cultures.** Transcriptional factor IRF-1 acts as a critical component of the type I IFN-dependent pathway that promotes the induction of IL-12 p55 transcription (26). Furthermore, IRF-1 has been described as cooperating with IRF-8 in the regulation of IL-12 p40 (29, 40) and IL-12 p35 transcription (27). To better understand the mechanisms responsible for the suppression of IL-12 p35 in MDDC infected with B. pertussis, we quantified IRF-1 and IRF-8 mRNA expression in MDDC infected with both B. pertussis strains. WT18323 infection induced low levels of IRF-1 and was unable to induce IRF-8 mRNA expression (Fig. 4). However, in response to ACT^-18HS19 infection, MDDC up-regulated both the transcription factors (Fig. 4). The addition of exogenous t-cAMP concomitantly to ACT^-18HS19 in MDDC cultures down-regulated IRF-1 and IRF-8 mRNA expression to levels similar to those induced by WT18323 strain (Fig. 4).

IRF-1 and IRF-8 transcription is induced upon type I IFN-α/β-mediated STAT-1 phosphorylation (37), and endogenous IFN-β has been recently proposed as a master regulator of IL-12 p35 via IRF-1 and possibly IRF-8 induction (15). Thus, IFN-β mRNA expression was assessed in MDDC infected with both strains. The results obtained indicated a lower IFN-β mRNA expression after infection with WT18323 than with ACT^-18HS19 infection (Fig. 4). Also in this case, t-cAMP addition to ACT^-18HS19-infected MDDC restored inhibition of IFN-β mRNA expression.

**B. pertussis ACT^-18HS19-infected MDDC drive a strong Th1-oriented polarization of immune response.** B. pertussis-infected MDDC induce a Th1 polarization of naive T cells, even in the absence of IL-12 p70 production (11), a key cytokine...
in Th1 polarization (39). We here compared the polarization capacity of CM obtained from WT18323- or ACT/H1100218HS19-infected MDDC. CM from untreated cells were used as a control. When naive T cells were cultured in the presence of ACT/H1100218HS19-CM, the number of IFN-γ-producing T cells was remarkably increased while IL-4-producing T cells were decreased compared to naive T cells cultured in the presence of WT18323-CM (Fig. 5). These results underline a stronger Th1 orientation of the immune response induced by B. pertussis ACT/H1100218HS19 with respect to WT18323, in line with the capacity of the former strain to induce Th1-polarizing cytokines, in particular IL-12 p70.

DISCUSSION

In the present study, we more deeply analyzed the suppression of IL-12 p70 production induced by B. pertussis infection in MDDC (11) and its impact on the polarization of the host immune response. The working hypothesis started from the notion that ACT inhibits LPS-driven IL-12 p70 production in human and murine DC (3, 34). Therefore, ACT/H18HS19, a strain defective in ACT activity (25), was used in parallel with its parental strain (WT18323) to infect MDDC. This approach led us to better understand the modulatory ability exerted by B. pertussis on MDDC, particularly the role played by intracellular cAMP accumulation induced by ACT.

The differential ability of the two B. pertussis strains to induce intracellular cAMP through ACT activity had no relevant effects on phenotypic maturation and allogeneic T-cell stimulation of MDDC. Recent studies indicate that ACT stimulation leads to an up-regulation of the maturation markers in MDDC in human DC (3) and mouse DC (34). Our data suggest that the ACT activity is not critical for the further induction of MDDC maturation induced by B. pertussis. This is probably due to the influence of other virulence factors, such as pertussis toxin, which is pivotal in keeping maturation markers at high levels of expression (2, 38). These data are in agreement with our previous results showing that phenotypic maturation and antigen presentation are triggered in MDDC mainly by epitopes which are also expressed by heat-inactivated bacteria and ruling out a major role played by associated enzymatic activities of virulence factors (11).
We demonstrate here that ACT activity has a central role in the modulation of IL-12 protein family expression by MDDC. These cytokines are heterodimeric encoded proteins and represent important physiological regulators in vivo for cellular immune response to microbial infections. Coordinated expression of the two genes is crucial for induction of the bioactive cytokine and for the appropriate induction of immune responses in timing, location, and magnitude (39). Only infection with the ACT/H11002 strain induced detectable levels of IL-12 p70 production in MDDC. This depends on the capacity of the ACT/H11002 strain to induce p35 mRNA expression compared to WT18323 strain.

A key role of IL-12 p35 in the regulation of IL-12 p70 secretion by DC has recently been described. Liu and colleagues demonstrated that cooperation between IRF-1 and IRF-8 is crucial for IL-12 p35 induction through binding to an IRF-8 response element in the human IL-12 p35 promoter (27). Gautier and coworkers described an autocrine-paracrine IFN-β-dependent pathway pivotal in inducing IRF-1/IRF-8-mediated IL-12 p35 transcription (15).

The observed IFN-β inhibition by B. pertussis infection may be dependent on the inhibition of the IRF-1-dependent positive feedback loop which plays a crucial role in fostering IFN-β production (37). Interestingly, our data agree with the observation that, in human macrophages, cAMP-mediated signaling inhibits the activation of the Jak-1/STAT-1 transduction pathway (10) that has been described as crucial for mediation of the IFN-β-dependent IL-12 p35 induction in human MDDC (15).

Our data indicate that ACT activity also reduces the expression of IL-23 by affecting both p40 and p19 gene expression but to a lesser extent with respect to the inhibition of p35 subunit. Thus, the final result is a shift from the expression of IL-23 only in WT18323-infected MDDC to the expression of either IL-12 and IL-23 in ACT/H11002-infected MDDC. Consequently ACT/H11002-infected MDDC possess a more pronounced Th1 polarization ability than WT18323-infected MDDC. cAMP intoxication induced by WT18323 in MDDC upon infection reduces the host ability to induce a Th1 protective immune response, representing a possible escape mechanism of the bacterium. These data are partially in accordance with that reported by Schnurr and colleagues, who described the inhibition of IL-12 p70 but the enhancement of IL-23 in response to cAMP elevation in MDDC (35). Boyd and colleagues recently demonstrated in mice that enzymatically active ACT promotes the induction of Th2 polarization and regulatory T cell activities in vivo (7). Indeed, we collected evidence that the loss of ACT activity in B. pertussis MDDC infection supports a stronger Th1 immune response induction. We were not able to detect any Th2 and/or T regulatory po-

FIG. 4. ACT°HS19 infection enhances IRF-1, IRF-8, and IFN-β mRNA expression in MDDC. Quantitative RT-PCR for IRF-1, IRF-8, and IFN-β gene expression by MDDC was performed at the indicated time points. Cells were left untreated (none) or infected with the WT18323 or ACT°HS19 strain, the latter in presence or absence of d-cAMP (100 µM). mRNA transcripts levels were expressed as relative increases over those in unstimulated MDDC at 5 h. Results of one representative experiment of three performed are shown.

FIG. 5. ACT°HS19 infection primes MDDC to induce a strong Th1 polarization of naive T cells. Naive T lymphocytes were cultured in the presence of PHA and with CM (50% vol/vol) obtained from MDDC left untreated (none) or infected with the WT18323 or ACT°HS19 strain for 24 h. On day 12, T cells were washed and restimulated with PMA-ionomycin in the presence of brefeldin A for IFN-γ and IL-4 intracellular staining. Numbers in each quadrant indicate the percentages of positive cells. Results of one representative experiment of three performed are shown.
larization ability of *B. pertussis*-infected MDDC. The two experimental models are markedly different. Indeed, we performed in vitro MDDC infection with living bacteria expressing or not expressing ACT, while Boyd and colleagues administered the toxin in vivo. In addition, murine and human immune responses to *B. pertussis* differ in several aspects, including a differential response to acellular vaccine: i.e., primary vaccination induces a response and potentiate and cytokine (type I interferon, IL-12 p70, and IL-23) production through a cAMP-dependent pathway. J. Leukoc. Biol. 66:1718–1725.

INHIBITS IL-12 p35 VIA ACT 2837

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