Anthrax Edema Toxin Cooperates with Lethal Toxin to Impair Cytokine Secretion during Infection of Dendritic Cells

Jean-Nicolas Tournier, Anne Quesnel-Hellmann, Jacques Mathieu, Cesare Montecucco, Wei-Jen Tang, Michèle Mock, Dominique R. Vidal and Pierre L. Goossens


http://www.jimmunol.org/content/174/8/4934

**References**

This article cites 45 articles, 25 of which you can access for free at:
http://www.jimmunol.org/content/174/8/4934.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Anthrax Edema Toxin Cooperates with Lethal Toxin to Impair Cytokine Secretion during Infection of Dendritic Cells

Jean-Nicolas Tournier, Anne Quesnel-Hellmann, Jacques Mathieu, Cesare Montecucco, Wei-Jen Tang, Michèle Mock, Dominique R. Vidal, and Pierre L. Goossens

Bacillus anthracis secretes two critical virulence factors, lethal toxin (LT) and edema toxin (ET). In this study, we show that murine bone marrow-derived dendritic cells (DC) infected with B. anthracis strains secreting ET exhibit a very different cytokine secretion pattern than DC infected with B. anthracis strains secreting LT, both toxins, or a nontoxicogenic strain. ET produced during infection selectively inhibits the production of IL-12p70 and TNF-α, whereas LT targets IL-10 and TNF-α production. To confirm the direct role of the toxins, we show that purified ET and LT similarly disrupt cytokine secretion by DC infected with a nontoxicogenic strain. These effects can be reversed by specific inhibitors of each toxin. Furthermore, ET inhibits in vivo IL-12p70 and IFN-γ secretion induced by LPS. These results suggest that ET produced during infection impairs DC functions and cooperates with LT to suppress the innate immune response. This may represent a new strategy developed by B. anthracis to escape the host immune response. The Journal of Immunology, 2005, 174: 4934–4941.

Copyright © 2005 by The American Association of Immunologists, Inc.

The Journal of Immunology

Received for publication July 27, 2004. Accepted for publication January 25, 2005.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

A recent study has shown that purified LT disrupts the functions of LPS-activated DC (9). Most studies examining ET and LT effects on macrophages and DC were conducted with purified toxins. The specific roles of ET and LT produced during infection have not yet been analyzed. In this study, we used different mutants of B. anthracis expressing ET and/or LT to investigate the effects of each toxin.

We show that murine bone marrow-derived DC exhibit very different cytokine secretion patterns when infected with B. anthracis strains secreting ET, LT, or both, and when infected with a LT/ET-negative strain. Our results suggest that ET cooperatively suppresses the immune response with LT, representing a new strategy for escaping the host’s immune response.
Materials and Methods

**Generation of mouse bone marrow-derived DC**

DC were generated from proliferating bone marrow progenitors from male BALB/c (H-2d) and C57BL/6 (H-2b) mice, as described previously (18). DC were negative for monocyte-macrophage (CD14), B cell (CD45R/ B220), and granulocyte (Gr-1) marker, and exhibited a phenotype of myeloid immature DC (CD11c⁺, CD11b⁺, CD4⁻, CD8α⁻, MHC-Ⅱ⁻, MHC-Ⅱlow, CD40⁻, CD80low, CD86⁻/low).

**A. anthracis strains**

The following A. anthracis strains were studied: the parental Sterne strain 7702 (pXO1⁻), single- mutant derivatives RP10 Δeff and RP9 Δγα that produce PA-EF and PA-LF, respectively (19), and double-mutant RP42 Δeff/Δγα producing PA only (20).

**Infection of DC**

DC were seeded at 1.5 × 10⁶ cells/ml in RPMI 1640 (Sigma-Aldrich) supplemented with 5% FCS (Invitrogen Life Technologies) and 2 mM l-glutamine (Sigma-Aldrich). Cells were incubated with A. anthracis spores for 1 h at a multiplicity of infection (MOI) of 20 and then washed twice and resuspended in medium containing 2.5 μg/ml gentamicin (Invitrogen Life Technologies) to kill any remaining extracellular bacteria (21). Culture supernatants were sampled 17 h later. Unstimulated DC were used as negative controls, and LPS-stimulated DC (1 μg/ml) were used as a positive control.

**Preincubation of DC with toxins, MAPK inhibitors, or forskolin**

DC were incubated with PA (1 μg/ml), and/or EF, and/or LF at various concentrations (0.1–40 ng/ml; all three components from List Biological Laboratories) for 2 h at 37°C. Spores of strain RP42 (LT⁻/ET⁻) were then added for 1 h at an MOI of 20. Cells were washed twice. Fresh culture medium containing gentamicin (2.5 μg/ml) was added for the remaining time. Culture supernatants were sampled 17 h later.

To correlate LT-induced effects with disruptions of MAPK pathways, DC were preincubated for 2 h with SB203580 (10 μM; Sigma-Aldrich), an inhibitor of the p38 MAPK pathway; and/or with PD98059 (10 μM; Calbiochem), an inhibitor of the ERK1/2 pathway; or with forskolin from Coleus Forskohlii (100 μM; Sigma-Aldrich), an activator of adenyl cyclase. Spores of strain RP42 (LT⁻/ET⁻) were then added for 1 h at an MOI of 20. Cells were washed twice, fresh culture medium containing gentamicin (2.5 μg/ml) and each inhibitor were added for the remaining time. Culture supernatants were sampled 17 h later.

**Western blotting**

DC were preincubated with PA (1 μg/ml) and LF at 40 ng/ml (List Biological Laboratories) for 2 h at 37°C. DC were then stimulated by spores of RP42 strain (LT⁻/ET⁻). After 5 min of incubation, cells were washed twice with cold PBS and lysed on ice for 15 min with a cell extraction buffer (20 mM Tris-HCl, pH 7.5, 1 mM sodium EDTA, 1 mM EGTA, 1% Triton X-100, 1 mM DTT, 0.05% Nonidet P-40, 0.27 M sucrose, 1 mM Na₃VO₄, 10 mM β-glycerophosphate, 5 mM NaF, and Complete Mini protease inhibitor (Roche)). After sonification and centrifugation, total protein concentration of the lysates was determined by use of MicroBCA Protein Assay Reagent (Pierce). Total protein (25 μg) was resolved on 10% SDS-PAGE gels and transferred to nitrocellulose membranes (Bio-Rad).

Protein detection was performed with polyclonal Abs directed against phospho-p38 MAPK, total p38 MAPK, or total p42/p44 (ERK1/2) MAPK (all from Cell Signaling Technology). Nitrocellulose membranes were also labeled with anti-HEK-3 polyclonal Abs (Santa Cruz Biotechnology). Bands were visualized with appropriate secondary HRP-conjugated Abs (Jackson Immunoresearch Laboratories) and SuperSignal West Pico chemiluminescent substrate (Pierce).

**Toxin inhibition assays**

For ET, 3 μM adefovir dipivoxil (Bis-POM-PMEA) (obtained from C. Gibbs, Gilead Sciences, Foster City, CA); 9-2-[bis[[(pivaloyloxy)methoxy]methyl]phosphinyl]-methoxyethyl]adenine was preincubated with DC for 5 h at 37°C. PA (1 μg) plus 40 or 10 ng of EF was added. Cells were then treated, as described above, except that 3 μM Bis-POM-PMEA was added to the cells after washing. Controls consisted of Bis-POM-PMEA or PA + EF alone.

For LT, 1 μg of PA plus 40 or 10 ng of LF were preincubated with 20 μM (-) epigallocatechin-3-gallate (EGCG): ((2R,3R)-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-1[H]-benzopyran-3,5-tiol-3-(3,4,5-trihydroxybenzoate)) (Calbiochem) for 10 min at 37°C. Cells were then added and processed, as described above. Controls were incubated with EGCG or PA + LF only.

**DC intracellular staining of spores**

DC were incubated with Sterne 7702 strain spores for 1 h at 37°C and then washed twice. Cells were stained with PE-coupled anti-CD11c mAb (HL3; BD Biosciences), fixed in 1% paraformaldehyde, and then permeabilized with 0.1% saponin-1% BSA in PBS before being incubated with FITC-labeled anti-BclA mAb (487G12; obtained from P. Sylvester, Institut Pasteur, Paris, France). Cells were fixed in 1% paraformaldehyde and analyzed on a FACSVantage (BD Biosciences).

**Transmission electron microscopy**

DC were incubated with Sterne 7702 spores, as described above, and analyzed after 30 min. Cells were fixed for 3 h in 3% glutaraldehyde in 0.1 M phosphate buffer and postfixed for 1 h with 1% OsO₄ in 0.1 M cacodylate buffer. The pellet was embedded in epoxy resin and sectioned for transmission electron microscopy.

**Analysis of cell surface phenotype and cell viability**

Cells were labeled with the following Abs conjugated with FITC or PE (all from BD Biosciences): HL3 anti-CD11c, 3/23 anti-CD40, 6-10A1 anti-CD80, GL1 anti-CD86, AMS-32.1 anti-I-A^d, and AF6-120.1 anti-I-A^d. Ten micrometers of 7-aminoactinomycin D (7-AAD) (Sigma-Aldrich) were added to the cells just before analysis to assess cell viability.

**Cytokine measurement in supernatants and intracellular cytokine staining**

IL-10, IL-12p70, and TNF-α were measured in 18-h DC culture supernatants using ELISA kits (R&D Systems).

**In vivo IL-12p70 and IFN-γ release after LPS challenge**

BALB/c and C57BL/6 mice (6–12 wk old) were injected i.p. with 250 μl of PBS alone, or PBS containing PA and/or LF (10 μg each). One hour later, 250 μg of LPS from Salmonella typhimurium (Sigma-Aldrich) in 100 μl of PBS was injected i.p. IL-12p70 and IFN-γ concentrations in serum collected 4 h later were measured by ELISA (R&D Systems).

**Results**

**Spores are efficiently phagocytosed by DC, but toxin secretion does not impair their maturation**

Given the strain-related susceptibility of several immune cells such as macrophages to LT-induced cell death (14, 22), DC were derived from BALB/c-sensitive and C57BL/6-resistant mice. DC were infected with spores of the Sterne strain (7702) or Sterne-derived mutants that express ET (RP10), LT (RP9), or neither (RP42) upon germination. Within 1 h of infection, phagocytosed spores were visible in >80% of DC, as assessed by electron and optical microscopy analysis (Fig. 1, A and B), or by flow cytometry after intracellular staining with a spore-specific anti-BclA mAb (Fig. 1C). DC viability at 18 h of infection was not affected by toxins secreted by the different mutants, as assessed by 7-AAD staining (survival >89% for C57BL/6- and 85% for BALB/c-derived DC). We observed that infection with spores of A. anthracis induced the up-regulation of costimulatory CD40, CD80, and CD86 molecules that were unaffected by toxin secretion either for C57BL/6- or BALB/c-derived DC (Fig. 2, A and B, respectively). This shows that during an infection, secreted ET and LT do not affect DC maturation and survival.

**ET and LT secreted during A. anthracis infection differentially impair cytokine secretion by DC**

We evaluated TNF-α, IL-10, and IL-12p70 cytokines produced by DC after exposure to spores of the different mutants. RP42 spores (LT⁻/ET⁻) induced a high level of the three cytokines tested. However, striking differences were observed with respect to the ET⁺/LT⁻ and LT⁺/ET⁻ strains (Fig. 3). TNF-α secretion was inhibited by the RP10 (ET⁺/LT⁻), but also by 7702 (LT⁺/ET⁻).
FIGURE 1. Spores are efficiently phagocytozed by DC. A, Transmission electron micrograph of DC incubated with 7702 (LT'/ET') spores for 30 min. B, May-Grünwald-Giemsa staining of DC after 1 h of interaction with 7702 (LT'/ET') spores. C, Intracellular staining for exosporium BclA protein of DC incubated for 1 h with 7702 (LT'/ET') spores.

FIGURE 2. Secretion of toxins by germinating spores does not impair DC maturation. A and B, Pheno-type analysis of C57BL/6 (A) and BALB/c (B)-derived DC incubated with RP42 (LT'/ET'), 7702 (LT'/ET'), RP9 (LT'/ET'), or RP10 (LT'/ET') spores for 18 h. Values showing percentage of positive cells measured over surface are indicated by horizontal bars. Results shown are representative of three different experiments.
and RP9 (LT+/ET−) strains in BALB/c- and C57BL/6-derived DC (p < 0.005). The inhibitory effects of the two toxins were cumulative, as observed during infection with the 7702 (LT+/ET+) strain vs RP10 (ET+/LT−) or vs RP9 (LT+/ET−) (p < 0.05 for BALB/c- and C57BL/6-derived cells, respectively). IL-10 secretion was strongly inhibited by the LT+/ET+ and LT+/ET− strains in C57BL/6- and BALB/c-derived DC, but not by the ET+/LT− strain. IL-10 was even significantly increased by ET+/LT− strain in BALB/c-derived DC. In contrast, IL-12p70 was inhibited by the ET+/LT− and LT+/ET− strains in both C57BL/6- and BALB/c-derived DC. Interestingly, the LT+/ET− strain induced a significant increase in IL-12p70 secretion in C57BL/6 only and no change in BALB/c-derived DC.

Purified ET and LT differentially impair DC cytokine secretion

The results above suggest that ET and LT produced during infection exert a differential effect on DC cytokine secretion. To confirm this observation, we investigated the effects of purified toxins on DC cytokine secretion. Preliminary studies showed that ET and LT alone did not induce any cytokine secretion by DC (data not shown). Therefore, we preincubated DC with different concentrations of purified ET and LT for 2 h, and added spores of the LT+/ET− mutant for 1 h. Cytokine secretion was assayed 17 h later. DC survival was not affected at 18 h by ET and LT (75% survival), except for BALB/c-derived DC at the highest LT concentration (26% survival at 40 ng/ml LT) (Fig. 4). As expected, purified ET and LT target the same cytokines as during infection with spores from toxin-secreting strains. On DC from both BALB/c and C57BL/6 mice, ET induced a dose-dependent decrease in TNF-α and IL-12p70 secretion, whereas it did not modify IL-10 secretion. An IL-12p70 decrease was also observed in the presence of forskolin, suggesting that these effects were due to the increase of cAMP (Fig. 5A). On both C57BL/6- and BALB/c-derived DC (for BALB/c, at nonlethal LT concentrations, i.e., below 40 ng/ml), we observed that LT induced a dose-dependent decrease in IL-10 and TNF-α secretion, whereas it did not in IL-12 secretion.

To investigate the mechanisms of LT action on C57BL/6 DC, we analyzed the p38 and ERK1/2 MAPK pathways in RP42-infected DC treated or not with LT. Infection with RP42 (LT+/ET−) spores induced a rapid phosphorylation of both p38 and ERK1/2.
The p38 MAPK inhibitor SB203580 significantly inhibited the IL-12p70 secretion observed in these conditions of stimulation, whereas the ERK inhibitor PD98059 had no significant effect, and both inhibitors were additive (Fig. 5A). We assessed the presence of MEK-3, a MAPK kinase known to phosphorylate downstream p38 and to be crucial to IL-12p70 secretion (23). Interestingly, when DC were incubated with 40 ng/ml LT, we observed that MEK-3 was not cleaved (Fig. 5B). We further investigated the phosphorylation of p38 and ERK1/2 MAPK in these RP42-stimulated and LT-treated DC; we observed that LT inhibited ERK1/2, but not p38 MAPK phosphorylation (Fig. 5C).

**ET and LT inhibitors restore DC cytokine secretion**

We next confirmed that the effects of ET and LT on DC were due to the enzymatic activities of EF and LF using the following specific chemical inhibitors: adefovir dipivoxil (Bis-POM-PMEA) (24) and EGCG, respectively (25). We found that preincubation with Bis-POM-PMEA restored TNF-α and IL-12p70 secretions by ET-treated DC to normal levels (Fig. 6A). Similarly, preincubation of LT at high concentration with EGCG inhibited the LT-induced mortality of BALB/c-derived DC and restored secretion of TNF-α and IL-10 in DC derived from both mouse strains (Fig. 6, B and C). The affinity of adefovir diphosphate, the active cellular metabolite of Bis-POM-PMEA for EF-calmodulin complex, is 10- to 500-fold higher than for mammalian host adenyl cyclase (24); EGCG has been shown to have no toxicity even at concentrations exceeding 100 μM in rats and humans (25). The low concentrations used in these experiments and the absence of cell toxicity suggest a specific interaction between inhibitors and their respective targets.

The results obtained with purified toxins confirm our cell infection experiments and show that ET and LT target different cytokine secretion pathways and that these effects are related to the enzymatic activity of EF and LF.

**ET impairs IL-12p70 and IFN-γ secretion in vivo**

Finally, to demonstrate the effects of ET and LT in an established in vivo model of IL-12 production (26), we examined the ability of ET and LT to inhibit IL-12p70 and IFN-γ secretion in mice challenged systemically with the strongest IL-12p70 inducer, LPS (Fig. 7). We found that mice treated with ET alone, or in combination with LT before the LPS challenge, show significantly lower serum concentrations of IL-12p70 and IFN-γ in both C57BL/6 and BALB/c mice, compared with mice treated with LPS alone. Interestingly, LT alone had no statistically significant effect. These findings strongly suggest that in vivo the role of ET may be critical during the initial steps of immune response by impairing early IL-12p70 and IFN-γ secretion.

**Discussion**

Our data are the first to show that ET as well as LT secreted during *B. anthracis* infection severely impairs DC cytokine secretion, potentially disrupting the host’s innate immune response. ET inhibits IL-12p70 and TNF-α secretion, whereas LT inhibits IL-10 and TNF-α secretion. ET and LT have a cumulative effect on TNF-α secretion.

The role of ET in anthrax pathogenesis is less known than LT, although it has been shown that ET-deleted mutants are less virulent than their parental strains (19). A previous study has shown that purified ET differentially regulates the production of TNF-α and IL-6 by LPS-induced human monocytes (4). The present work is the first to explore the effects of ET on DC, the pivotal cells implied in the control of innate and adaptive immune response. The effects of ET on cytokine secretion are very similar to the effects of two other bacterial toxins: cholera toxin (CT) (26–28).

![FIGURE 5. RP42 strain induces p38 and ERK1/2 MAPK phosphorylation, whereas LT inhibits the ERK1/2 MAPK pathway only. A, DC derived from C57BL/6 mice were preincubated with SB203580 (a p38 inhibitor), and/or PD98059 (an ERK inhibitor), or forskolin (a toxin that induces an increase of intracellular cAMP) for 2 h and infected with RP42 (LT /ET -) spores. IL-12p70 secretion was measured 18 h after the infection. Data show mean cytokine concentrations (±SD) representative of three independent experiments. t test: *, p < 0.05; **, p < 0.005 compared with RP42 strain. B and C, DC derived from C57BL/6 mice were preincubated or not with LT at 40 ng/ml for 2 h, and further stimulated by spores of RP42 strain for 5 min. Cells were then lysed for MEK-3 (B), total and phospho-p38, and total and phospho-ERK1/2 (C) analysis by Western blot. Control cells were either stimulated by RP42 only, or not stimulated. Data show one representative experiment from three independent experiments.](http://www.jimmunol.org/doi/pdf/10.4049/jimmunol.1700067)
and pertussis toxin (PT) (29). PT and CT are AB toxins that are clearly distinct from ET. ET has an adenylyl cyclase activity, while CT activates \( \alpha \)-subunits of G proteins (Gs), a GTP-binding protein that stimulates adenylyl cyclase, leading to enhanced cAMP intracellular level. PT ADP ribosylates the G\( \alpha \) subunit of Gi protein and thus inactivates it. Adenylyl cyclase activity is enhanced by PT only if a positive stimulus for the induction of adenylyl cyclase is present. It has been shown that cAMP-elevating agents suppress DC function through the increase of IL-10 (30) and the inhibition of IL-12 secretion (31, 32). The similar effects of these toxins and ET on DC cytokine secretion may be linked to their intracellular target, the cAMP, as suggested by the forskolin-induced inhibition of IL-12 secretion. Moreover, ET-induced IL-12 inhibition observed in vitro was correlated to our in vivo experiments using a LPS-triggered IL-12 secretion model.

Our study shows that LT inhibits IL-10 and TNF-\( \alpha \) production by DC. The effects of LT on cytokine secretion and their consequences on immunity have been previously addressed (33, 34). Our results on the effects of LT on the proinflammatory cytokine TNF-\( \alpha \) are consistent with previous reports on macrophages (10, 11) and DC (9). However, we observed significant differences in

FIGURE 6. ET and LT inhibitors restore DC cytokine secretion. A. Inhibition of the effects of EF on DC by Bis-POM-PMEA. DC from C57BL/6 (■) and BALB/c (□) mice were preincubated with Bis-POM-PMEA and ET and then infected with RP42 (LT/ET) spores. Data show the mean cytokine concentrations in 18-h culture supernatants (±SD) representative of three independent experiments. Control cells were incubated with RP42 spores (LT/ET) alone or together with Bis-POM-PMEA. \( t \) test; *, \( p < 0.05 \); **, \( p < 0.005 \) compared with RP42 strain. B. Inhibition of the effects of LF on DC by EGCG. LF was preincubated with EGCG before addition to DC from C57BL/6 (■) and BALB/c (□) mice. Cells were then infected with RP42 (LT/ET) spores. Data show the mean cytokine concentrations (±SD) in culture supernatants after 18 h; representative results from three independent experiments. Control cells were incubated with RP42 spores (LT/ET) alone or together with EGCG. \( t \) test; **, \( p < 0.005 \) compared with RP42 strain. C. Effect of EGCG on the viability of BALB/c-derived DC incubated with LT, as assessed by light microscopy.
terms of DC maturation and IL-12 secretion with a previous study (9). These apparent discrepancies on DC reactivity to LT could be due to several factors. In our study, DC viability after LT treatment was not affected at the time of supernatant sampling for cytokine measurements (i.e., 18 h), except at high LT concentration (40 ng/ml) for BALB/c-derived DC. Even in this case, early evaluation of cell viability (i.e., 8 h) showed no mortality (data not shown). In contrast, we stimulated DC with a physiologically relevant pathogen-associated molecular pattern (i.e., live B. anthracis spores), in contrast to LPS used in most studies to trigger cell activation (9–13). This could notably modify the intracellular signaling pathway because peptidoglycans of Gram-positive bacteria are known to activate the TLR2 pathway, whereas LPS of Gram-negative bacteria stimulates TLR4 (35). Recent studies have clearly shown that the cytoplasmic tail of TLR2 and TLR4 differs functionally, and elicits receptor-specific signaling and inflammatory responses. TLR4 activates both MyD88-independent pathway, resulting in activation of IFN-regulatory factor 3 and MyD88-dependent pathways, whereas TLR2 facilitates the activation of the MyD88-dependent pathway only (36). The alternate activation of TLR2/TLR4 pathways could explain differences in cytokine secretion and/or apoptosis induction, as previously reported in human DC and murine macrophages (37, 38).

In our study, we showed that RP42 spores induced rapid phosphorylation of both p38 and ERK1/2. Interestingly, we observed that infection with spores of LT-secretting strains induced an increase of IL-12p70 secretion by C57BL/6-derived DC, which are considered as resistant to LT-induced cell death (14). LT at 40 ng/ml induced an inhibition of ERK1/2 phosphorylation. In contrast, p38 MAPK phosphorylation was not affected. Similar results have previously been reported on macrophages derived from C57BL/6 mice activated by LPS (39). Taken together, these results fit with data concerning the regulation of IL-12p70 secretion through a balance between ERK and p38 MAPK (40–42). An inhibition of ERK1/2 and an activation of the p38 pathway have indeed been shown to induce IL-12p70 secretion. Furthermore, the absence of MEK-3 cleavage suggests that despite the ability of LT to cleave a large range of MEK, it may cleave MEK-3 with less efficiency than other MEK targets upstream ERK1/2 at physiological concentrations. These differential cleavages may thus induce a sensitive balance between ERK and p38 MAPK pathways; this balance could be of great significance for pathogen survival, because MEK are crucial downstream molecules involved in TLR signaling (43). Local LT concentration may thus be a critical factor that should be integrated into relevant physiological studies. Moreover, our in vivo results, which clearly indicate that LT does not inhibit secretion of LPS-triggered IL-12 and IFN-γ (a cytokine induced by IL-12), are strongly consistent with our in vitro results.

Another point to consider is that B. anthracis physiologically secretes both ET and LT during infection. A significant contribution of this study is the assessment of the effects of ET and LT alone, or in association, during an infection. The infection results are perhaps the most relevant in terms of a physiological perspective. With respect to the cytokine released in the supernatant, the effects of the association of ET and LT depend on the cytokine tested: ET has a dominant effect on IL-12p70 secretion, whereas LT is dominant on IL-10 secretion, and ET and LT have a cumulative effect on TNF-α. Taken together, they show that both toxins cooperate to impair DC cytokine secretion.

These data are the first to show that ET and LT target distinct DC cytokine secretion pathways and that ET inhibits in vitro and in vivo the production of IL-12, a cytokine that is critical for innate resistance and adaptive immunity through Th1 activation. Our results shed new light on previous studies reporting that ET and LT cooperate during intoxication (44) or infection in vivo (19, 45). An understanding of how B. anthracis escape innate and adaptive immunity may lead to novel therapeutic strategies to improve the efficiency of immune responses toward anthrax.

Acknowledgments
We thank F. Desor and G. Brochier for their technical support.

Disclosures
The authors have no financial conflict of interest.

References


