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Anthrax Lethal Toxin Blocks MAPK Kinase-Dependent IL-2 Production in CD4\(^+\) T Cells\(^1\)

Hui Fang, Ruth Cordoba-Rodriguez, Carla S. R. Lankford, and David M. Frucht\(^2\)

Anthrax lethal toxin (LT) is a critical virulence factor that cleaves and inactivates MAPK kinases (MAPKKs) in host cells and has been proposed as a therapeutic target in the treatment of human anthrax infections. Despite the potential use of anti-toxin agents in humans, the standard activity assays for anthrax LT are currently based on cytotoxic actions of anthrax LT that are cell-, strain-, and species-specific, which have not been demonstrated to occur in human cells. We now report that T cell proliferation and IL-2 production inversely correlate with anthrax LT levels in human cell assays. The model CD4\(^+\) T cell tumor line, Jurkat, is a susceptible target for the specific protease action of anthrax LT. Anthrax LT cleaves and inactivates MAPKKs in Jurkat cells, whereas not affecting proximal or parallel TCR signal transduction pathways. Moreover, anthrax LT specifically inhibits PMA/ionomycin- and anti-CD3-induced IL-2 production in Jurkat cells. An inhibitor of the protease activity of anthrax LT completely restores IL-2 production by anthrax LT-treated Jurkat cells. Anthrax LT acts on primary CD4\(^+\) T cells as well, cleaving MAPKKs and leading to a 95% reduction in anti-CD3-induced proliferation and IL-2 production. These findings not only will be useful in the development of new human cell-based bioassays for the activity of anthrax LT, but they also suggest new mechanisms that facilitate immune evasion by Bacillus anthracis. Specifically, anthrax LT inhibits IL-2 production and proliferative responses in CD4\(^+\) T cells, thereby blocking functions that are pivotal in the regulation of immune responses. The Journal of Immunology, 2005, 174: 4966–4971.

B. anthracis infection is primarily a disease of cattle, but humans can acquire the infection following contact with infected animal products or soil (1). Despite the relatively low numbers of naturally occurring infections in the United States (2), B. anthracis is a deadly and efficient bioterrorism agent, as recently demonstrated by the anthrax attacks in late 2001. Efforts are underway to develop and stockpile therapeutics for the treatment of established anthrax infection to confront this serious threat to public health (3). To this end, anthrax toxin components have been attractive therapeutic targets for investigational agents (4), because these bacterial proteins are critical virulence factors for B. anthracis. These toxin components include lethal factor (LF),\(^3\) edema factor (EF), and protective Ag (PA). Anthrax PA is essential for pore formation, allowing intracellular transport of the active toxin enzymes, EF, and LF. EF is an adenylate cyclase, whereas LF is a metalloprotease that inactivates MAPK kinases (MAPKKs) through cleavage at specific recognition sites (5–8).

The pathway for development of anthrax therapeutics for humans faces several technical challenges. For example, it will be necessary to perform pivotal efficacy testing in animal models of anthrax infection due to the low national incidence of naturally occurring anthrax infection in humans (9). For this reason, it is especially important to develop in vitro assays for anthrax toxin activity that most closely reflect the in vivo effects of the toxin during human infection. Assays that are widely used at present for anthrax LT are limited in that they are based on the species- and strain-specific action of the toxin to lyse and/or to inhibit proliferation of BALB/c-derived murine macrophage lines (10). Validated anthrax LT bioassays that are based on human cell parameters would fill a critical void in the development of anthrax therapeutics. Anthrax LT is known to be a potent modulator of cytokine signaling networks, either enhancing (11, 12) or inhibiting (7, 13, 14) production and/or extracellular release of proinflammatory cytokines from APCs depending on the stimulation conditions. Because MAPKK-dependent cytokine production is a feature common to a variety of cell types from anthrax-susceptible species, we investigated whether modulation of cytokine production by anthrax LT in non-APC could form an alternative basis for anthrax LT bioassays.

We report here that anthrax LT is a potent inhibitor of the MAPKK-dependent up-regulation of IL-2 production by Jurkat cells, a CD4\(^+\) T cell line. Moreover, anthrax LT blocks IL-2 production and IL-2-dependent proliferation by primary human CD4\(^+\) T cells following TCR stimulation. These findings not only provide the basis for the development of human cell-based bioassays for the activity of anthrax LT, but they also suggest that the direct effects of anthrax LT on CD4\(^+\) T cells could play a role in the pathogenesis of human infection.

Materials and Methods

Cell lines and culture conditions

Jurkat E6.1 cells were provided by the laboratory of Dr. Richard Siegel. Jurkat cells and purified human CD4\(^+\) T cells were cultured in RPMI 1640 complete medium containing 10% FBS (HyClone), 10 mM HEPES buffer, 1 mM sodium pyruvate (Quality Biological), 2 mM L-glutamine (Invitrogen Life Technologies), and 1% antibiotic/antimycotic (Sigma-Aldrich). Cell viability was assessed by trypan blue exclusion.

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\(^1\) The information presented here reflects the views of the authors and does not necessarily represent the policy of the U.S. Food and Drug Administration.

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\(^3\) Abbreviations used in this paper: LT, lethal toxin; EF, edema factor; PA, protective antigen; MAPKK, MAPK kinase; PLC, phospholipase C.
Reagents

Recombinant anthrax PA and LF were purchased from List Biological Laboratories and stored in 1:1 glycerol/water. Unless otherwise indicated, LT treatments were administered in excess at 2.5 μg/ml PA and 1 μg/ml LF. Ionomycin and PMA were prepared in DMSO and used at a dose of 5 nM and 50 ng/ml, respectively (EMD Biosciences). Recombinant IL-2, neutralizing anti-human IL-2, anti-human CD3, and anti-human CD28 Abs were obtained from R&D Systems. In-2-LF, a specific inhibitor of the protease activity of anthrax LT, was obtained from Calbiochem.

**CD4+ T cell purification**

Human CD4+ T cells were prepared from buffy coats from anonymous donors (Department of Transfusion Medicine, Clinical Center, National Institutes of Health, Bethesda, MD) using a two-step purification procedure. Briefly, the cell suspension was incubated for 1 h at room temperature with RosetteSep Human CD4+ Cell enrichment cocktail (StemCell Technologies) at a ratio of 20:1. The sample was then diluted with an equal volume of PBS with 2% FBS. CD4+ T cells were isolated by negative selection following separation through a Ficoll gradient (non-CD4+ cells were sedimented). Next, the CD4+ T cells were positively selected using MACS beads, following the manufacturer’s suggested protocol. Briefly, the cells were washed three times with PBS supplemented with 2% FBS, enumerated, and resuspended in MACS buffer. Anti-CD4 MACS beads were added at a ratio of 40 μl per 107 cells. Following column loading, column wash, and elution, the recovered cells were >99.5% CD4+ as assessed by flow cytometry.

**Western blotting**

For most experiments, centrifuged pellets generated from 1 to 2 x 107 cells were lysed on ice for 30 min in a buffer containing 20 mM Tris-HCl, 150 mM NaCl, 5 mM EDTA, 2.5 mM sodium pyrophosphate, 1 mM Na3VO4, 1% Triton X-100, and a protease inhibitor cocktail (Sigma-Aldrich). Western blotting was performed using standard techniques as previously described (12). The following primary Abs were used for Western blotting assays: anti-MEK1 (BD Biosciences); anti-MEK2 and anti-β-actin (Santa Cruz Biotechnology); anti-PLC-γ1, anti-phospho-PLC-γ1, anti-p44/42 MAPK, anti-phospho-p44/42 MAPK, anti-phospho-JNK, anti-JNK, anti-phospho-p38 MAPK, and anti-p38 MAPK (Cell Signalting Technology). HRP-conjugated anti-rabbit (Amersham Biosciences), anti-mouse (Amersham Biosciences), or anti-goat (Abcam) Abs were used as secondary antibodies.

**IL-2 ELISA assays**

Human IL-2 cytokine levels were determined using a commercial ELISA kit (R&D Systems). Unless otherwise indicated, cells were stimulated for 24 h. Cell-free supernatants were then obtained by centrifugation and were assayed neat or diluted according to the manufacturer’s protocol. Absorbance readings were performed using a microplate reader (Dynatech Laboratories). Each culture was assayed in duplicate or triplicate, with results averaged.

**MTT assays**

Jurkat cells were washed with PBS, resuspended at a concentration of 6.25 x 10^6 cells/ml in RPMI 1640 complete medium in 96-well plates (100 μl/well). Cells were treated with or without LT and incubated in a 5% CO2 humidified incubator at 37°C. At the indicated time points, cellular proliferation was measured by MTT using a commercial kit, following the manufacturer’s protocol (R&D Systems). Briefly, 10 μl of MTT reagent was added to each well. The cultures were further incubated for 2–4 h at 37°C until a purple precipitate was visible under the microscope. Detergent reagent was then added to the wells. The treated cultures were then incubated in the dark at 37°C for at least 3 h. Absorbance at 570 nm was measured using a spectrophotometer (Dynatech Laboratories). Each MTT assay was performed in triplicate.

**Tritiated thymidine incorporation assays**

Culture plates (96-well; Corning) were precoated with 1 μg/ml anti-CD3 and/or anti-CD28 (R&D Systems) at 4°C overnight. Following a PBS wash, 1.5 x 10^6 human CD4+ T cells were added to each well in a final volume of 100 μl of complete RPMI 1640. Cells were stimulated as indicated for 48 h at 37°C, pulsed with 1 μCi of tritiated thymidine (PerkinElmer Life and Analytical Sciences) overnight, and then processed using a harvester (PerkinElmer Wallac). Incorporated tritiated thymidine was measured using a 1205 Betaplate liquid scintillation counter (PerkinElmer Wallac). Each proliferation assay was performed in triplicate.

**Calcium mobilization assays**

Jurkat cells were incubated in RPMI 1640 complete medium with or without anthrax LT for 3 h. Cells were then harvested, centrifuged, washed, and resuspended in HBSS with 0.5% BSA (Sigma-Aldrich). Subsequently, 1 μg/mL (Calbiochem) was added to a final concentration of 1.8 μM, and this cell suspension was incubated at 37°C for 30 min with continuous agitation. The cells were then washed three times in loading buffer, and resuspended at a ratio of 4 x 10^6 cells/2 ml of buffer. The cell suspension was passed through a 70-μm filter before analysis. Following stimulation with supernatant from the C305 hybridoma, which expresses anti-Jurkat TCR mAb (16), the ratio of unbound/bound Ca2+ was measured on a Shimadzu RF 1501 spectrofluorophotometer (Mandel Scientific Company).

**Transfections and luciferase assays**

Jurkat cells (10^5) were transfected with 5 μg of NF-κB- or AP-1-responsive luciferase constructs (pNF-κB-Luc and pAP1-Luc; BD Biosciences) by electroporation as previously described (17). Cells were cultured overnight in RPMI 1640 complete medium (10^6 cells/10 ml) and then transferred to 24-well plates and treated in the presence or absence of a specific Jurkat TCR-activating mAb (C305), PMA, ionomycin, and/or anthrax LT (1 μg/ml LF + 2.5 μg/ml PA) for 5 h in RPMI 1640 complete medium (400 μl). After stimulation, the cells were centrifuged and the pellet lysed in luciferase reporter buffer (Promega). A 20-μl portion of each sample lysate was added to 100 μl of the luciferase assay reagent (Promega) and luminescence determined on a Microplate luminometer LB96V (Berthold Technologies).

**Results**

**Anthrax LT blocks MAPKK-dependent signaling in Jurkat cells**

Jurkat T cells, a human CD4+ T cell line, have proven to be a useful model in the characterization of TCR-dependent signaling pathways, including the MAPK pathways (ERK/JNK/p38). We investigated whether anthrax LT would cleave and inactivate MAPKs in Jurkat cells as it does in several other human primary cells and cell lines (5, 6). Lysates generated from Jurkat cells treated with anthrax LT were assayed by Western blotting for MEK-1 and MEK-2 in a time course experiment. Degradation of MEK-1 and MEK-2 was observed starting 30 min after anthrax LT treatment, with complete degradation of these two MAPKs between 2 and 3 h (Fig. 1A). Anthrax LT-dependent proteolysis of MAPKs correlated with the inhibition of signaling to their downstream MAPK targets (ERK, p38, and JNK). As shown in Fig. 1B, anthrax LT blocked both basal phosphorylation and anti-TCR-induced phosphorylation of p38 (Fig. 1B, top panel) and p44/p42 ERK (Fig. 1B, middle panel). In addition, anthrax LT entirely blocked basal and anti-TCR-induced phosphorylation of p46 JNK, and partially reduced basal levels of p54 JNK phosphorylation (Fig. 1B, bottom panel), similar to observations in anthrax LT-treated murine macrophages (18). A decrease was also observed in the anti-TCR- and PMA-induced activity of an AP-1 reporter construct in anthrax LT-treated Jurkat cells (Fig. 1C). As AP-1 is a downstream effector in MAPK/MAPK signaling pathways, our findings were all consistent with a specific action of anthrax LT to inactivate MAPKs in Jurkat cells, thereby disrupting downstream signaling.

**Jurkat cell proliferation, TCR-dependent calcium mobilization, and NF-κB activation are not affected by anthrax LT**

We next examined the effect of anthrax LT on a variety of functions of Jurkat cells in our culture conditions, because anthrax LT has been reported to have a pro-apoptotic effect on PBMC (14). As shown in Fig. 2A, there was minimal difference in the proliferation of Jurkat cells cultured in the presence or absence of anthrax LT.
Anthrax LT-treated Jurkat cells proliferated at rates greater than 94% of control untreated cultures for up to 3 days following treatment. Trypan blue exclusion testing of anthrax LT-treated Jurkat cells showed no decrease in the viability compared with untreated cells (H11022 95% normal viability following 48 h of treatment, not shown).

Subsequently, we examined the effect of anthrax LT on signal transduction in Jurkat cells in response to stimulation through the TCR. A time course experiment involving Western blotting with anti-phosphotyrosine (4G10) showed no difference in the pattern of phosphorylated proteins comparing anthrax LT-treated and untreated Jurkat cells treated with a TCR-specific mAb agonist (anti-TCR/C305 stimulation, 0/2/5/10 min, not shown). Similarly, as shown in Fig. 2B, anthrax LT did not affect the TCR-dependent phosphorylation of PLC-Π1, a signal transduction factor that is the nexus for several TCR-dependent signaling pathways, ultimately leading to mobilization of intracellular calcium stores and activation of NF-κB and AP-1. Anthrax LT treatment of Jurkat cells did

**FIGURE 1.** Blockade of MAPKK-dependent signaling in anthrax LT-treated Jurkat cells. A, Jurkat cell cultures were treated with anthrax LT for varying amounts of time as indicated. Total MEK-1 levels (top panel), MEK-2 levels (middle panel), and β-actin levels (loading control, bottom panel) were determined by Western blotting. B, Jurkat cells precultured in the presence or absence of anthrax LT were re-stimulated with C305 for 5 min. Levels of phospho-p38, phospho-ERK p44/42, and phospho-JNK p54/46 were assessed by Western blotting. Subsequently, levels of total p38, ERK p44/42, and JNK p54/46 were determined by reblotting these membranes. C, Jurkat cells transfected with an AP-1-responsive luciferase construct were precultured in the presence or absence of anthrax LT and re-stimulated with C305 or PMA for 5 h. Reporter activity was assayed in triplicate using a luminometer. Representative experiments from three (A, B, and C) separate experiments are shown. Error shown in C represents intraassay SD generated from triplicate samples.

**FIGURE 2.** Anthrax LT does not block parallel TCR signal transduction pathways. A, Jurkat cell cultures were cultured in the presence or absence of anthrax LT and proliferation rates assessed by MTT. B, Jurkat cells precultured in the presence or absence of anthrax LT were re-stimulated with C305 for varying amounts of time. Levels of pY783-PLC-Π1 (top panel) and total PLC-Π1 (reblot, bottom panel) were assessed by Western blotting. C, Jurkat cells pre-cultured in the presence or absence of anthrax LT were re-stimulated with C305 Ab and calcium mobilization assayed using Indo 1/AM. D, Jurkat cells transfected with an NF-κB-responsive luciferase construct were pre-cultured in the presence or absence of anthrax LT and re-stimulated with combined PMA and ionomycin for 5 h. Reporter activity was assayed in triplicate using a luminometer. Representative experiments from two (B and C) or three (A, D) separate experiments are shown. Error shown in A and D represents intraassay SD generated from triplicate samples.
not affect TCR-induced mobilization of calcium (Fig. 2C) or PMA/ionomycin-induced activity of a transfected NF-κB reporter construct (Fig. 2D), indicating that the toxin does not block the signal transduction pathways leading to these downstream effectors.

**Anthrax LT blocks IL-2 production in anti-CD3- and PMA/ionomycin-treated Jurkat cells**

We hypothesized that cellular functions of Jurkat cells that depend on MAPKK signaling could be used as indicators of anthrax LT activity. The production of IL-2 by Jurkat cells in response to stimulation by either anti-TCR (19) or by combined PMA and ionomycin (20), for example, has been shown to involve MAPK signaling. For this reason, we investigated whether anthrax LT inhibits the production of IL-2 by Jurkat cells stimulated through these pathways.

As shown in Fig. 3A, the production of IL-2 by anti-CD3-, PMA-, ionomycin-, or PMA/ionomycin-treated Jurkat cells was greatly reduced subsequent to pretreatment with anthrax LT. Moreover, the blockade of IL-2 production by anthrax LT could be completely overcome by an inhibitor of the proteolytic action of the toxin on MAPKKs in a dose-dependent manner (Fig. 3B, top and bottom panels), confirming the role of this pathway in the IL-2 production blockade. Anthrax LT had a dose-dependent effect on IL-2 production by stimulated Jurkat cells. As shown in Fig. 3C, this effect was detected at a dose as little as 1–10 ng/ml LF (PA in excess, top panel) and 25–250 ng/ml PA (LF in excess, bottom panel).

**FIGURE 3.** Anthrax LT blocks anti-TCR- and PMA/ionomycin-stimulated IL-2 production in Jurkat cells. A, Jurkat cells (2 × 10⁶ cells/ml) were cultured in the presence or absence of anthrax LT, anti-CD3, anti-CD28, PMA, and/or ionomycin as indicated for 24 h. Subsequently, IL-2 levels in culture supernatants were determined by ELISA. B (top panel), Jurkat cells (1 × 10⁶/ml) were cultured with PMA and ionomycin in the presence or absence of LT and varying amounts of a specific inhibitor of LT enzyme activity for 24 h in a dose-response experiment as shown. IL-2 levels in culture supernatants were then determined by ELISA. B (bottom panel), To demonstrate activity of the anthrax LT inhibitor, Jurkat cells were stimulated for 2 h with or without PA, LF, and/or the LT inhibitor (10 μM). Cell lysates were assayed by Western blotting with anti-total MEK-1 and anti-total MEK-2. C, Jurkat cells (1 × 10⁶/ml) were cultured in the presence or absence of a fixed amount of PA (2.5 μg/ml, top panel) or LF (1 μg/ml, bottom panel) and variable amounts of LF (top panel) or PA (bottom panel) as indicated in dose-response experiments. Cultures were stimulated with PMA and ionomycin for 24 h. Supernatants were assayed for IL-2 production by ELISA. Representative experiments from two (A and B) or three (C) separate experiments are shown. Error shown in A and B represents intraassay SD generated from triplicate samples. * none detected.

Anthrax LT inhibits proliferation and IL-2 production by CD4⁺ T cells stimulated through the TCR

We next investigated whether our findings in Jurkat cells could be extended to primary human CD4⁺ T cells. Human CD4⁺ T cells bound FITC-labeled PA in a specific manner (data not shown). Furthermore, as we had observed in Jurkat cells, anthrax LT treatment of primary CD4⁺ T cells led to the rapid proteolysis and reduction of MEK-1 and MEK-2 levels within 3 h (Fig. 4A, left panel). Anthrax LT suppressed MEK-1 and MEK-2 levels for up to 48 h, with or without anti-CD3 or anti-CD3/CD28 stimulation (Fig. 4A, right panel). In contrast to certain murine cell lines, treatment of purified human CD4⁺ T cells with anthrax LT was not associated with decreased viability in our experimental conditions (Fig. 4B). However, anthrax LT treatment did lead to a >95% blockade of anti-CD3- and PMA/ionomycin-induced proliferation (Fig. 4C). Subsequently, we examined the effect of anthrax LT on the CD28 costimulatory pathway. Stimulation with anti-CD28 alone did not induce proliferation, but high level proliferation was detected in CD4⁺ T cells stimulated with a combination of anti-CD3 and anti-CD28. These results indicated that CD28 signaling overcomes the specific TCR signaling blockade caused by anthrax LT treatment and further demonstrated that anthrax LT was permissive to cell survival and proliferation under certain conditions of stimulation (Fig. 4C).

In contrast to Jurkat cells, primary CD4⁺ T cells depend on IL-2 production for normal proliferation in response to TCR stimulation. As IL-2 is positively regulated by MAPK signaling (19), we investigated whether a blockade of IL-2 production was responsible...
FIGURE 4. Anthrax LT inhibits IL-2 production and proliferation in human CD4⁺ T cells. Purified human CD4⁺ T cells were cultured in the presence or absence of anthrax LT, anti-CD3, or anti-CD3/anti-CD28 as indicated. A. Total MEK-1, MEK-2, and β-actin levels (loading control) were determined by Western blotting. B. Cell viability was determined by propidium iodine staining and FACS analysis using the same experimental conditions shown in A. C. Proliferation of purified CD4⁺ cells cultured in the presence or absence of anthrax LT, anti-CD3, and/or anti-CD28 for 48 h was determined by measuring the incorporation of tritiated thymidine. D, IL-2 levels in the supernatants of CD4⁺ T cells (1 × 10⁶/ml) cultured in the presence or absence of anthrax LT, anti-CD3, and/or anti-CD28 were determined by ELISA. Representative experiments of a total of two (A and C) or three (D) separate experiments are shown. Error shown in C and D represents intraassay SD generated from triplicate samples. Data shown in B represents the results of three separate experiments, with interassay SD indicated. *, p < 0.05.

for the anthrax LT-induced proliferation blockade. To this end, we first examined the effect of the addition of exogenous IL-2 or neutralizing anti-IL-2 to anti-CD3-stimulated cultures. As shown in Fig. 4C, the proliferation rates in the combined IL-2/anti-CD3/anthrax LT-treated CD4⁺ T cell cultures were not different from those in the combined anti-IL-2/anti-CD3/anti-CD28 cultures. These data support a substantial role for IL-2 in the anti-proliferative effect of anthrax LT.

To directly address the question of the role of anthrax LT in modulating IL-2 production, human CD4⁺ T cells were stimulated with anti-CD3 and/or anti-CD28. Co-treatment of anthrax LT with anti-CD3-treated CD4⁺ T cells led to a 97% reduction in the induction of IL-2 to near basal levels (Fig. 4D). Stimulation through the CD28 pathway enhanced IL-2 production in anthrax LT/anti-CD3/anti-CD28-treated cultures, but did not fully correct the IL-2 production deficit (18.6 vs 69.4 ng/ml in anti-CD3/anti-CD28-treated cultures). Taken together these data demonstrate that anthrax LT inhibits TCR-dependent IL-2 production, but this effect is specific to the TCR signaling pathway and not due to a general cytotoxic effect. Through a yet undetermined mechanism, the CD28 signaling pathway partially overcomes this blockade.

Discussion

As the primary cell targets for infection by B. anthracis, the emphasis of anthrax research has logically centered on macrophages. One potential mechanism through which B. anthracis avoids being killed in macrophages is via the production of the anthrax LT. Anthrax LT protease cleaves MAPKKs (21), which are critical components of the signal transduction pathways required for the activation of macrophages in response to bacterial components and to key pro-inflammatory cytokines. By disabling these pro-inflammatory pathways, it has been proposed that B. anthracis is able to avoid containment by macrophages and, ultimately, overwhelms its host (22).

Although the role of macrophages as a target for anthrax LT is convincing, it is important to note that the critical role for MAPKK signaling is not restricted to macrophages, and that anthrax toxin receptors are widely expressed (23, 24). Recent studies have shown deleterious effects of the toxin not only on other types of APCs (e.g., dendritic cells) (25), but also on non-leukocytes such as human endothelial cells (26). Pro-apoptotic effects of anthrax LT treatment on unpurified human PBMC have been reported (14), but direct effects on purified T cells have not been previously demonstrated.

Our investigation of the effects of anthrax LT on purified CD4⁺ T cells or T cell lines clearly demonstrate that human CD4⁺ T cells are direct targets for anthrax LT. Although it has been reported recently that serum IL-2 levels are not affected by the treatment of rats with anthrax LT alone (27), the effects of anthrax LT on CD4⁺ T cells during TCR stimulation conditions have not been previously addressed. We find that the degradation of MAPKKs by anthrax LT renders human CD4⁺ T cells unable to respond normally to signaling through the TCR. Specifically, anthrax LT blocks the ability of human CD4⁺ T cells to make IL-2 in response to stimulation through their Ag receptors. Because IL-2 is a key cytokine that regulates T cell proliferation and cellular immunity (28), our data suggest that anthrax LT might have direct effects on the cells regulating adaptive immunity. The effects of anthrax LT on both innate and adaptive immune responses could act in synergy to paralyze overall immune responses in infected hosts. The combined effects of MAPKK signal transduction blockade in many types of cells, including non-immune cells, could be responsible for many of the late-stage sequelae of anthrax infection.

We have also shown that anthrax LT cleaves and inactivates MAPKKs in the human CD4⁺ T cell line, Jurkat. This, in turn,
leads to the specific blockade of the MAPKK signaling pathways in response to TCR stimulation and the dose-dependent inhibition of TCR-dependent IL-2 production. Moreover, the defect in MAPKK-dependent IL-2 production in Jurkat cells is evident upon stimulation with the combination of PMA and ionomycin. The specific dose-dependent action of anthrax LT to block IL-2 production in Jurkat cells by either of these stimuli is a basis for measuring the activity of anthrax LT activity in human cells. These findings, therefore, could facilitate the development of convenient and relevant tools for assessing potency of therapeutics that target anthrax LT.

In addition, the selective action of anthrax LT on MAPKK signaling suggests that anthrax LT could be a useful reagent in assessing the role of MAPKCs in other signaling pathways, including the CD28 pathway. CD28 signaling reversed some of the anthrax LT-induced blockade in TCR-dependent IL-2 production through an undetermined mechanism. It will be important in future experiments to determine whether the CD28 activation pathway augments IL-2 production in anthrax LT-treated cells via residual MAPKK signaling (29) or via a MAPKK-independent mechanism. Experiments examining whether anthrax LT has differential effects on naive vs effector CD4+ T cells are also warranted, because naive and effector CD4+ T cells differ in their co-stimulatory requirements. Moreover, in addition to IL-2, it will be important to determine whether other cytokines would be suitable markers for the activity of anthrax LT. In this regard, anthrax LT reduces the production of IFN-γ and IL-4 in human CD4+ T cells treated with anti-CD3 in the presence or absence of anti-CD28, though the mechanisms for these effects have not been determined (data not shown).

The recent use of anthrax as a bioterrorism agent has highlighted the critical need to understand and then to combat the mechanisms that this pathogen uses to avoid control by the human immune system. Our data support a model in which anthrax LT has a direct effect on suppressing production of IL-2, a central cytokine in adaptive immune responses. These data not only have important implications for understanding the pathogenesis of anthrax infection, but they also establish the basis for developing human cell-based bioassays for evaluating potential therapeutics.

Note added in proof—S. R. Paccine and colleagues very recently showed (30) that the recent use of anthrax as a bioterrorism agent has highlighted the critical need to understand and then to combat the mechanisms that this pathogen uses to avoid control by the human immune system. Our data support a model in which anthrax LT has a direct effect on suppressing production of IL-2, a central cytokine in adaptive immune responses. These data not only have important implications for understanding the pathogenesis of anthrax infection, but they also establish the basis for developing human cell-based bioassays for evaluating potential therapeutics.

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Disclosures
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