Anthrax toxins suppress T lymphocyte activation by disrupting antigen receptor signaling

Silvia Rossi Paccani,[1] Fiorella Tonello,[3] Raffaella Ghittoni,[1,2] Mariarita Natale,[1] Lucia Muraro,[3] Mario Milco D'Elios,[4] Wei-Jen Tang,[5] Cesare Montecucco,[3] and Cosima T. Baldari[1]

[1]Department of Evolutionary Biology and [2]Department of Clinical Medicine and Immunological Sciences, Policlinico Le Scotte, University of Siena, 53100 Siena, Italy
[3]Department of Biomedical Sciences, University of Padua, 35121 Padua, Italy
[4]Department of Internal Medicine and Immunoallergology, University of Florence, 50134 Florence, Italy
[5]Ben-May Institute for Cancer Research, University of Chicago, Chicago, IL 60637

Anthrax is an infection caused by pathogenic strains of *Bacillus anthracis*, which secretes a three-component toxic complex consisting of protective antigen (PA), edema factor (EF), and lethal factor (LF). PA forms binary complexes with either LF or EF and mediates their entry into host cells. Although the initial phases of bacterial growth occur in the lymph node, the host fails to mount an effective immune response. Here, we show that LT and ET are potent suppressors of human T cell activation and proliferation triggered through the antigen receptor. Both LT and ET inhibit the mitogen-activated protein and stress kinase pathways, and both toxins inhibit activation of NFAT and AP-1, two transcription factors essential for cytokine gene expression. These data identify a novel strategy of immune evasion by *B. anthracis*, based on both effector subunits of the toxic complex, and targeted to a key cellular component of adaptive immunity.

CORRESPONDENCE
Cosima T. Baldari:
baldari@unisi.it

Anthrax is an infection caused by pathogenic strains of *Bacillus anthracis*. After entering the body though skin abrasions, ingestion, or inhalation, *B. anthracis* spores are phagocytosed by macrophages that migrate to regional lymph nodes. There, the spores germinate and become vegetative bacteria that lyse the macrophage, multiply in the lymphatic system, and enter the bloodstream, causing septicemia and toxemia and eventually leading to death of the host (1). *B. anthracis* secretes a binary A-B toxin composed of a single B protomer, protective antigen (PA), and two alternative A proteins, lethal factor (LF) and edema factor (EF). PA interacts with different cell surface receptors and, after proteolytic activation, self-associates to form membrane-inserting heptamers that bind LF and EF (2–4). PA + LF (lethal toxin [LT]) and PA + EF (edema toxin [ET]) complexes enter surface rafts (5) and are internalized into acidic endosomal compartments. Low pH triggers a conformational change that results in LF and EF translocation into the cytosol of the host cell (2–4). LF is a zinc metalloprotease that cleaves mitogen-activated protein kinase kinases (MAPKK; references 6–8), thereby interfering with the MAPK cascade, a crucial component in signaling by surface receptors controlling cell proliferation and survival. EF is a calcium/calmodulin-dependent adenylylcyclase that catalyzes massive production of cAMP, with ensuing alteration of intracellular signaling pathways (9).

Although manifestations of advanced disease are believed to be caused by anthrax toxin, host–pathogen interactions occurring in the lymph node are as yet largely elusive. LT blunts the release of proinflammatory mediators by macrophages and dendritic cells and promotes their apoptosis, thereby preventing phagocyte recruitment and bacterial clearance (10, 11). Furthermore, LT impairs dendritic cell function and interferes with initiation of adaptive immune responses (12). To date, the outcome of *B. anthracis* infection on T lymphocytes, the central regulators of adaptive immunity, has not been addressed, although these cells can be exposed to the toxin in the lymph node, and several steps in the signaling cascade initiated by the TCR may be potentially dis-

The Journal of Experimental Medicine

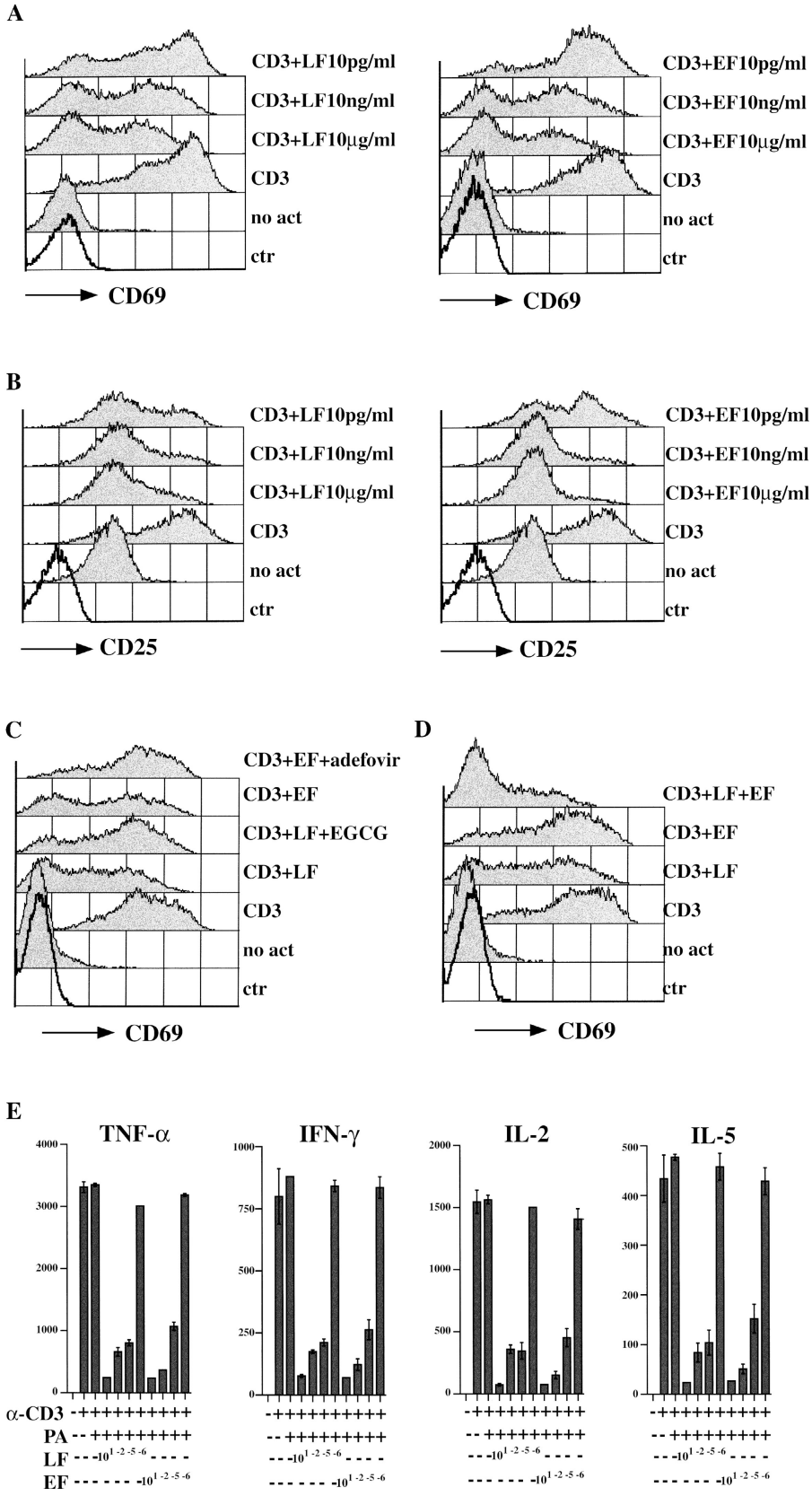

Figure 1. Anthrax toxins suppress T cell activation. Flow cytometric analysis of CD69 (A) or CD25 (B) expression on PBLs activated for 16–24 h by CD3 cross-linking in the presence or absence of 200 ng/ml PA and the indicated concentrations of either LF or EF. (C) CD69 expression on PBLs ac-

rupted by the activities of LF and EF (13–15). Here, we have investigated the outcome of T lymphocyte exposure to anthrax toxins.

RESULTS AND DISCUSSION

Commitment to T cell activation is characterized by the temporally regulated expression of genes encoding proteins that include transcription factors, cell surface receptors, and cytokines. The end of the commitment phase is marked by expression of IL-2 and IL-2R, which initiate an autocrine proliferation loop resulting in clonal expansion of antigen-specific T cells (16). To determine the effect of LT or ET on T cell activation, human PBLs were treated for 6 h with PA or PA in combination with varying amounts of LF or EF. After washing, cells were stimulated by TCR/CD3 ligation for 16–24 h. Trypan blue exclusion showed that neither toxin affected cell viability to a significant extent, even at the highest concentration used (not depicted). Surface expression of CD69, an early activation marker, and of CD25, the high affinity subunit of the IL-2 receptor, was measured by flow cytometry. Both LT and ET inhibited the expression of both CD69 (Fig. 1 A) and CD25 (Fig. 1 B) in a dose-responsive fashion. No effect was observed when cells were treated with PA, LF, or EF alone (not depicted). The specificity of these activities was confirmed by using selective inhibitors of LF and EF. Epigallocatechin-3-gallate (EGCG), a specific LF inhibitor (17), and adefovir dipivoxil, a specific EF inhibitor (18), selectively reversed the effect of LF and EF, respectively (Fig. 1 C and not depicted). Remarkably, when LT and ET were used together at the lowest concentration, which alone affected only weakly CD69 expression, a significant inhibition was observed, indicating a synergism of the two toxins (Fig. 1 D). The effect of anthrax toxins on cytokine production was assessed by quantitating the levels of IFN-γ, IL-2, IL-5, and TNFα in the culture supernatants of cells treated as described before and incubated for 30 h. As shown in Fig. 1 E, both LF and EF profoundly inhibited cytokine expression, further underlining the suppressive effect of anthrax toxins on T cell activation.

Long-term effects of the anthrax toxins on T cell proliferation were assayed by flow cytometric analysis of PBLs treated with LT or ET for 6 h and loaded, after washing, with the fluorescent vital dye, CFSE, which binds irreversibly to cellular proteins, followed by activation by TCR/CD3 cross-linking. TCR-dependent T cell proliferation was first detectable 48 h after activation (not depicted) and, by 72 h, a significant proportion of T lymphocytes was dividing, with up to three rounds of division. LT, and to an even larger extent ET, markedly inhibited cell proliferation (Fig. 2).

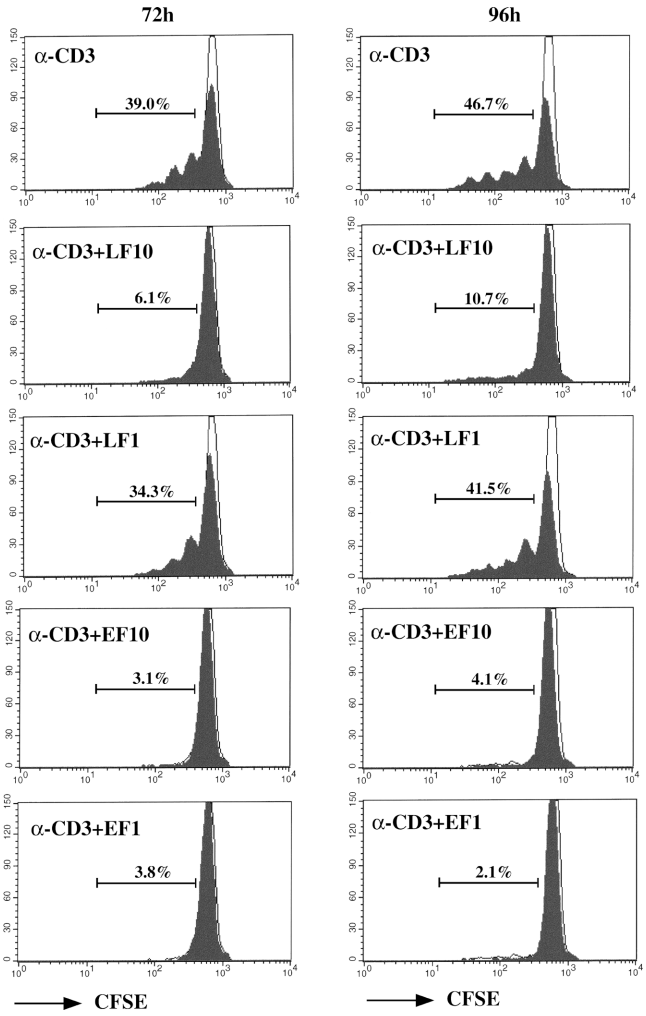

Figure 2. Suppression of T cell proliferation by anthrax toxins. Flow cytometric analysis of CFSE-labeled PBLs stimulated for 72 and 96 h by CD3 cross-linking (α-CD3) in the presence or absence of 200 ng/ml PA and 10 ng/ml (LF10, EF10) or 1 ng/ml (LF1, EF1) LF or EF. CFSE fluorescence was analyzed on gated $CD3^+$ cells. CFSE staining in unstimulated $CD3^+$ cells is shown as an unshaded histogram. The percentage of $CD3^+$ cells having undergone proliferation is indicated. Data from a representative experiment are shown ($n \geq 3$). For each experiment, proliferation was monitored at 24, 48, 72, 96, 120, and 148 h.

LF cleaves MAPKKs, including MEK1/2 and MKK3/4/6/7 (6–8), which are the intermediate members of the MAP and stress kinase cascades initiated by the small GTPases Ras and Rho/Rac/cdc42, respectively (19). Cleavage occurs within the NH_2-terminal proline-rich region preceding the kinase domain, thus disrupting a sequence involved in the protein–protein interactions required for activation of their downstream MAPK targets (20, 21). The effect of LT on the in-

tivated for 16 h by CD3 cross-linking in the presence or absence of 200 ng/ml PA and 10 ng/ml LF or EF, in combination with 10 μM EGCG or 5 μM adefovir dipivoxil. EGCG or adefovir dipivoxil alone did not affect CD69/CD25 expression either on quiescent or activated cells (not depicted). The inhibitory effect of LF on CD69/CD25 expression was not affected by adefovir dipivoxil, nor the effect of EF by EGCG (not depicted). (D) CD69 expression on PBLs activated for 16 h by CD3 cross-linking in the presence or absence of 200 ng/ml PA and 10 ng/ml LF or 10 pg/ml EF, either alone or in combination. Representative experiments are shown ($n \geq 3$). (E) Quantitation of cytokines (pg/ml) in the supernatants of PBLs activated for 30 h by CD3 cross-linking in the presence or absence of 200 ng/ml PA and the indicated concentrations of LF or EF (μg/ml) ($n = 2$). Error bars, SD.

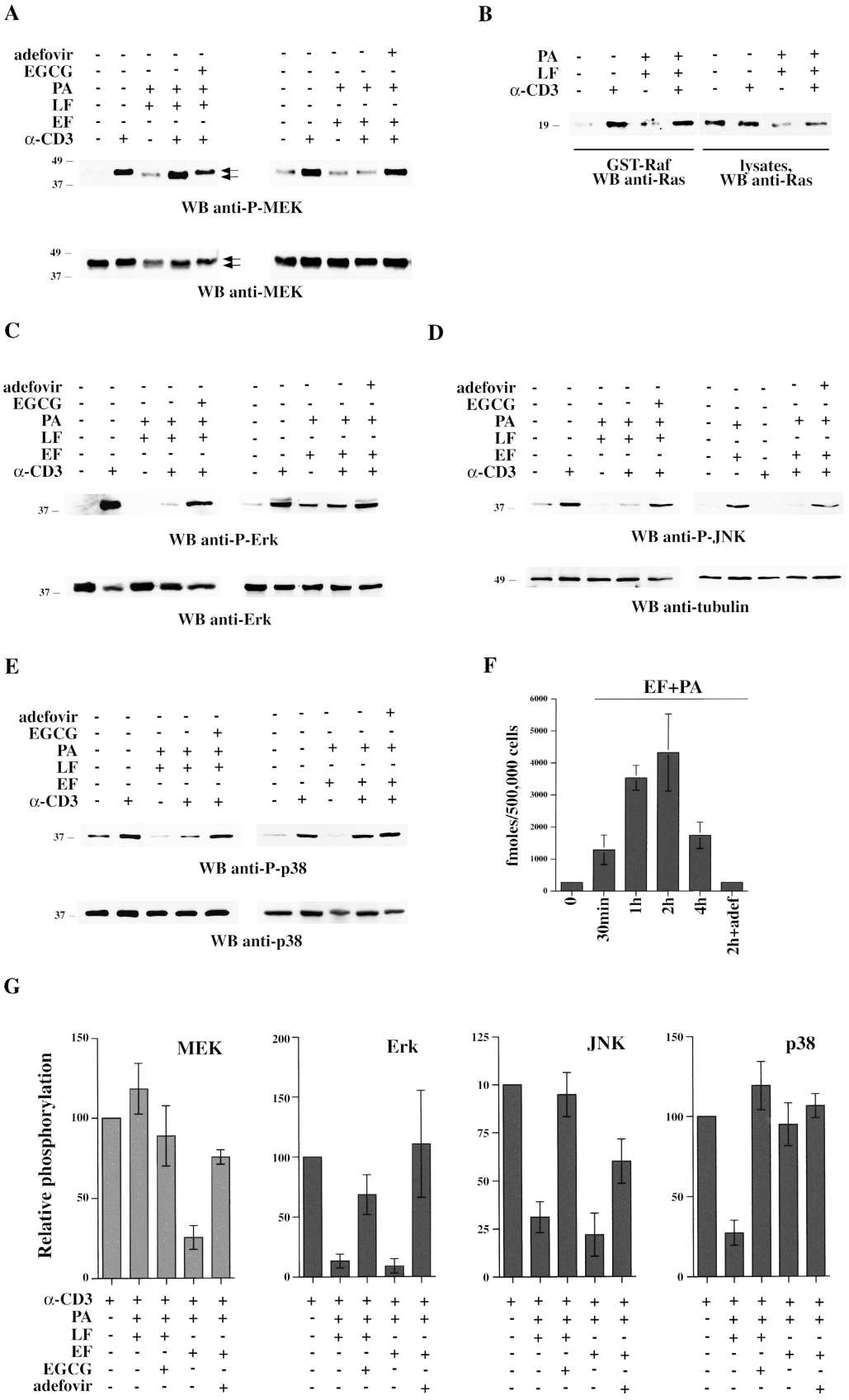

Figure 3. Anthrax toxins disrupt MAP and stress kinase activation. Immunoblot analysis of postnuclear supernatants from PBLs activated for 5 min by CD3 cross-linking (α-CD3) in the presence or absence of 0.2–2 μg/ml PA and 1–10 μg/ml LF or EF, in combination with 10 μM EGCG or 5 μM

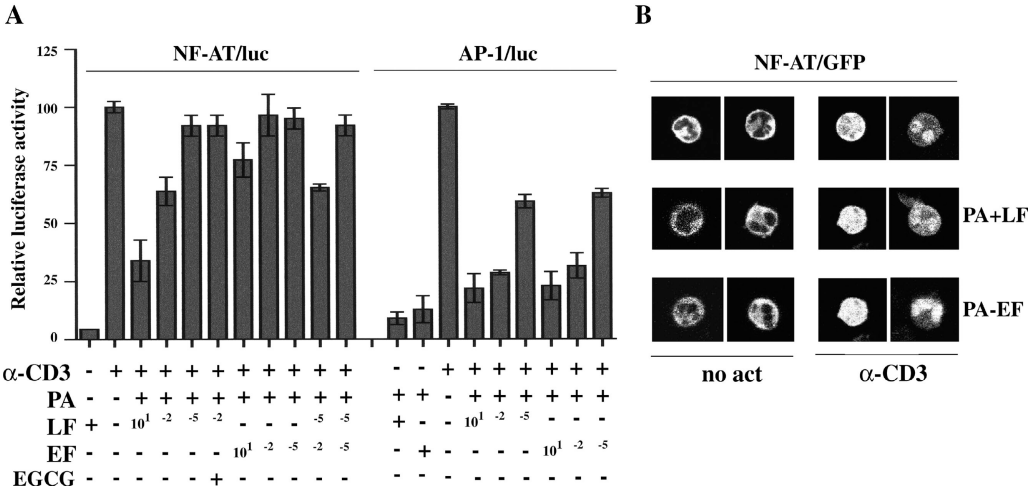

Figure 4. Inhibition of TCR-dependent NFAT and AP-1 activation by anthrax toxins. (A) Relative luciferase activity in NF-AT/luciferase reporter Jurkat cells (left) or Jurkat cells transiently transfected with an AP-1/luciferase reporter (right), activated by CD3 cross-linking in the presence or absence of 200 ng/ml PA and the indicated concentrations of LF or EF and, where indicated, 10 μM EGCG. The data are expressed as percentage of luciferase activity induced by CD3 cross-linking (100%) ($n \geq 3$ for NFAT, $n = 3$ for AP-1). Error bars, SD. (B) Confocal microscopy of Jurkat cells transiently transfected with a NFAT/GFP expression construct, either unstimulated (0) or activated by CD3 cross-linking for 30 min, in the absence or presence of 200 ng/ml PA and 1 μg/ml LF or EF. Percentage of cells showing nuclear staining in a representative experiment were as follows: no activation, 0%; CD3XL, 72.5%; LT, 9.4%; ET, 4.0%; CD3XL + LT, 68.0%; CD3XL + ET, 71.9% (no. of GFP^+ cells scored ≥50).

tegrity and activation of MEK1/2 was assessed. PBLs were treated for 6 h with either LT or ET, washed, and activated by TCR/CD3 cross-linking for 5 min. Cells were lysed, and postnuclear supernatants were subjected to immunoblot analysis using anti-MEK1/2 antibodies. Fig. 3 A shows that PBL treatment with LT resulted in the conversion of MEK1/2 to a higher electrophoretic mobility form consistent with removal of a NH_2-terminal, ~800 Da fragment. ET had no effect, and the shift in apparent MW was inhibited by EGCG, which blocks the proteolytic cleavage of MEKs by LF (17), but not by adefovir dipivoxil (Fig. 3 A and not depicted). In agreement with these results, immunoblot analysis of the stripped filters with an antibody that specifically recognizes the NH_2 terminus of MEK2 showed that the MEK2-specific immunoreactivity was significantly reduced in LT-treated PBLs, but not in PBLs treated with LT in the presence of EGCG, nor in ET-treated PBLs (not depicted). Probing the same lysates with antibodies against phosphorylated, active MEK1/2 showed that MEK1/2 phosphorylation was not impaired by LT (Fig. 3, A and G), consistent with the fact that MAPKK cleavage does not affect the site of interaction with Raf, its upstream kinase, nor its phosphorylation sites (22). Furthermore, Ras activation was not affected, as shown by pulldown assays using a GST-Raf fusion, which interacts with GTP-bound, active Ras (Fig. 3 B). Alternatively, activation of Erk, the MAPK downstream of MEK, was profoundly inhibited by LT. This effect was selectively reversed by EGCG, but not by adefovir dipivoxil (Fig. 3, C and G, and not depicted). Furthermore, in agreement with the capacity of LF to cleave a wide panel of MAPKKs (8), activation of the stress-kinases JNK and p38, which is mediated by MKK4/7 and MKK3/6, respectively (19), was also blocked by LT (Fig. 3, D, E, and G).

Interestingly, although ET did not affect the integrity of MEK1/2, it significantly reduced its activation (Fig. 3, A and G), as well as Erk activation (Fig. 3, C and G). PBL treatment with ET resulted in increased intracellular cAMP, which was blocked by adefovir dipivoxil (Fig. 3 F). The PKA-dependent inhibitory effect of cAMP on the activation of Raf (23), the serine-threonine kinase upstream of MEK, is likely to underlie the inhibition of MEK activation by ET. MEK/Erk inhibition by ET was reversed by adefovir dipivoxil but not by EGCG (Fig. 3, A, C, and G, and not depicted). These results highlight MEK as a point of convergence of the immunosuppressive activities of LT and ET. Of note, TCR-dependent JNK activation (Fig. 3, D and G), but not p38 activation (Fig. 3, E and G), was also impaired

adefovir dipivoxil. Immunoblots were performed with antibodies against the phosphorylated, active forms of MEK (A), Erk (C), JNK (D), or p38 (E). Filters were stripped and reprobed with anti-MEK (A), Erk (C), tubulin (D), or p38 (E) antibodies. Arrows indicate the full-length and cleaved forms of MEK (A). The inhibitory effects of LF were not affected by adefovir dipivoxil, nor the effect of EF by EGCG (not depicted). Neither EGCG nor adefovir dipivoxil had any effect on MAP or stress kinase activity in untreated, non-stimulated or anti-CD3–stimulated cells (not depicted). Representative experiments are shown ($n \geq 3$). (B, left) Immunoblot analysis using anti-Ras mAb of in vitro binding assays to agarose-bound GST-Raf of postnuclear supernatants from PBLs activated as described before in the presence or absence of 200 ng/ml PA and 10 μg/ml LF. Total cell lysates separated on the same gel are shown (right). (F) Quantitation of cAMP in lysates of PBLs treated with 2 μg/ml PA and 1 μg/ml EF, in the presence or absence of 5 μM adefovir dipivoxil. Error bars, SD. (G) Densitometric analysis of data from multiple experiments on the effects of LT and ET on Erk, JNK, p38, and MEK phosphorylation, in the absence or presence of EGCG or adefovir dipivoxil. The results are shown as the percentage of the phosphorylation levels of each kinase in anti-CD3–stimulated PBLs (100%) after normalization to the respective controls.

in cells treated with ET, suggesting a selectivity in the inhibitory activity of ET for specific MAP/stress-kinase cascades.

MAP kinases link triggering of surface receptors to gene transcription (13, 19). The transcription factor NFAT plays a crucial role in the transcriptional regulation of several cytokine-encoding genes, including the genes for IL-2 and IL-2R (24). NFAT is composed of a nuclear subunit, AP-1, which is activated by MAPKs, and a cytosolic subunit, which translocates into the nucleus to assemble with AP-1 after dephosphorylation by the phosphatase calcineurin. The latter is activated by the elevation in intracellular $[Ca^{2+}]_i$ triggered by TCR engagement (24). The effect of anthrax toxins on TCR-dependent NFAT activation was determined using a reporter Jurkat T cell line stably transfected with a construct encoding luciferase under the control of a trimer of the NFAT binding site on the IL-2 gene promoter. Consistent with the requirement for MAP and stress kinases in NFAT activation (24, 25), LT inhibited NFAT–driven luciferase expression in a dose-dependent fashion, and this effect was prevented by EGCG (Fig. 4 A). Inhibition of NFAT activation by LT resulted, at least in part, from AP-1 inhibition by the toxin, as shown in transient transfection experiments using an AP1/luciferase reporter (Fig. 4 A). However, LT did not affect the Ca^{2+}–calcineurin pathway. Indeed, LT had no effect on TCR-dependent NFAT translocation to the nucleus, as determined by confocal microscopy of Jurkat cells transiently transfected with a NFAT–GFP fusion (Fig. 4 B). Furthermore, LT did not affect $[Ca^{2+}]_i$ flux triggered by TCR/CD3 ligation in PBLs (not depicted).

TCR-dependent NFAT activation was also inhibited by ET, albeit only at the highest dose used (Fig. 4 A). This effect appears dependent on the capacity of ET to block Erk and JNK, which control AP-1 activation (19), as AP-1–driven luciferase expression was also inhibited by ET (Fig. 4 A), whereas NFAT/GFP nuclear translocation was not affected by the toxin (Fig. 4 B). Of note, a combination of PA with 10 ng/ml EF and 10 pg/ml LF, which alone did not affect NFAT–driven luciferase expression, resulted in significant inhibition of NFAT activation (Fig. 4 A), indicating a synergism of the two toxins. Interestingly, as opposed to NFAT, AP-1 activation was inhibited also at lower ET concentrations (Fig. 4 B). The lack of effect of ET on the activation of p38, which is implicated in the control of NFAT, but not AP-1 activity (25), is likely to underlie the different sensitivity of the two transcription factors to ET.

The present results identify T lymphocytes as a novel cellular target of anthrax toxins and show that the intracellular activities of both LT and ET converge in these cells on the signaling cascade initiated by the TCR, resulting in suppression of cell activation and proliferation. Although the causal role of the anthrax toxic complex in the advanced phase of infection has been largely elucidated, the failure of the host to mount an effective immune response is as yet elusive. Adaptive immunity is initiated in the lymph node, where vegetative bacteria are released from infected macrophages and can be taken up by dendritic cells, processed, and presented to T cells. LT has been shown recently to interfere with MAPK signaling in dendritic cells, thereby suppressing their maturation and capacity to prime adaptive responses (12). The data reported here highlight T lymphocytes as direct cellular targets of both LT and ET. The two toxins cooperatively inhibit T cell activation at very low concentrations, similar to those expected in the early stages of infection. By suppressing the activation of both dendritic cells and T cells, which together are responsible for initiating adaptive immune responses, *B. anthracis* has evolved a highly effective strategy of immune evasion.

MATERIALS AND METHODS

Cells, reagents, and antibodies. Cells included the T lymphoma Jurkat line and a stably transfected Jurkat line expressing luciferase under the control of a trimer of the distal NFAT binding site on the IL-2 promoter (26). PBLs were purified from the whole blood of healthy donors by density gradient centrifugation and subsequent depletion of monocytes by adherence. Phospho-specific antibodies recognizing the active forms of MEK1/2, Erk1/2, JNK, and p38, and anti-MEK1/2 antibodies were obtained from Cell Signaling Technology; anti-p38 and anti-Erk antibodies, as well as an anti-MEK2 antibody specific for its NH_2 terminus, were obtained from Santa Cruz Biotechnology, Inc.; antitubulin mAb was obtained from Amersham Biosciences. Fluorochrome-labeled anti-CD3, anti-CD69, and anti-CD25 mAb were obtained from Becton Dickinson. IgG from OKT3 (anti-CD3; American Type Culture Collection) hybridoma supernatants were protein G purified. Unlabeled secondary antibodies were from Cappel and peroxidase-labeled antibodies were obtained from Amersham Biosciences. Sepharose-conjugated GST-Raf and anti-Ras mAb were from Upstate Biotechnology. PA and LF were expressed and purified as described previously (27). The EF gene from the plasmid pMMA187 (28) was PCR amplified using the following primers: 5′-AAAGGATCCTCCATGAATGAACATTACACTGAG-3′ (forward) and 5′-AAAGAGCTCTTATTTTTCATCCATAATTTT-TTGG-3′ (reverse). The PCR fragment obtained was digested with BamHI and SacI and inserted in pRSET A (Invitrogen). The cloned sequence was confirmed by DNA sequencing. The protein was expressed as NH_2-terminal His-tag fusion in BL21 DE3 (Novagen Inc.) and purified by Ni-charged Hitrap chelating (Amersham Biosciences). EGCG was purchased from Sigma-Aldrich and adefovir dipivoxil was synthesized and supplied by Gilead Sciences Inc. Cells were plated at 5×10^6 cells/ml in RPMI 1640 supplemented with 7.5% FCS complete medium, added with LT and/or ET, and incubated at 37°C for 6 h. EGCG was preincubated with LT or ET for 15 min at 37°C before addition to the cells. Cells were preincubated with adefovir dipivoxil at 37°C for 2–6 h before addition of LT or ET.

Activations, in vitro binding assays, immunoblots, luciferase assays, and cytokine and cAMP measurements. For immunoblot and in vitro binding experiments, activations by TCR/CD3 ligation were performed by incubating cells with anti-CD3 mAb and secondary antibodies for 5 min at 37°C as described previously (29). For analysis of CD69/CD25 and luciferase expression, cells were activated by CD3 cross-linking on secondary antibody-coated plates (29) and processed for flow cytometry 16–24 h after activation. NFAT/luciferase reporter Jurkat cells were collected 16 h after activation and processed for luciferase assays (29). In vitro binding assays and immunoblots were performed as described previously (30) and quantitated by laser densitometry (Kodak Digital Science Electrophoresis Documentation and Analysis System 120). Intracellular cAMP was quantitated by enzyme-linked immunoassay (Biotrak EIA; Amersham Biosciences). Cytokines were measured using the Human Cytokine ELISA kit obtained from Bender MedSystems.

Transfections, confocal microscopy, and flow cytometry. Jurkat cells were transiently transfected with the plasmid pEGFP/NFAT-1D (31)

or an AP-1/luciferase reporter using a modification of the DEAE/dextran procedure as described previously (29). Confocal microscopy was performed on a confocal microscope (Microsystems; Leica). CD3, CD69, and CD25 surface expression was quantitated by flow cytometry using fluorochrome-labeled mAb. Samples were processed using a FACScan flow cytometer (Becton Dickinson). T cell proliferation was measured by flow cytometric analysis of CFSE-labeled cells, counterstained with anti-CD3 mAb as described previously (30). Cells were analyzed 24–172 h after stimulation. Intracellular flux of calcium ions was measured as described previously (29) using FF-fluo-4 (Molecular Probes).

The authors wish to thank J.L. Telford for critical reading of the paper, S. Grassini for technical assistance, C. Gibbs (Gilead Sciences Inc.) for the kind gift of adefovir dipivoxil, and V. Di Bartolo for generously providing the AP-1 reporter.

This work was supported by grants from AIRC, MIUR (FIRB), EU (Epipepvac to C.T. Baldari and the Anthrax Euronet to C. Montecucco), and the National Institutes of Health (to W.-J. Tang).

The authors have no conflicting financial interests.

Submitted: 4 August 2004
Accepted: 8 November 2004

REFERENCES

1. Dixon, T.C., M. Meselson, J. Guillemin, and P.C. Hanna. 1999. Anthrax. *N. Engl. J. Med.* 341:815–826.
2. Ascenzi, P. P. Visca, G. Ippolito, A. Spallarossa, M. Bolognesi, and C. Montecucco. 2002. Anthrax toxin: a tripartite lethal combination. *FEBS Lett.* 531:384–388.
3. Mock, M., and T. Mignot. 2003. Anthrax toxins and the host: a story of intimacy. *Cell. Microbiol.* 5:15–23.
4. Collier, R.J., and J.A. Young. 2003. Anthrax toxin. *Annu. Rev. Cell Dev. Biol.* 19:45–70.
5. Abrami, L., C. Liu, P. Cosson, S.H. Leppla, and F.G. van der Goot. 2003. Anthrax toxin triggers endocytosis of its receptor via a lipid raft-mediated clathrin-dependent process. *J. Cell Biol.* 160:321–328.
6. Duesbery, N.S., C.P. Webb, S.H. Leppla, V.M. Gordon, K.R. Klimpel, T.D. Copeland, N.G. Ahn, M.K. Oskarsson, K. Fukasawa, K.D. Paull, and G.F. Vande Woude. 1998. Proteolytic inactivation of MAP-kinase-kinase by anthrax lethal factor. *Science.* 280:734–737.
7. Vitale, G., R. Pellizzari, C. Recchi, G. Napolitani, M. Mock, and C. Montecucco. 1998. Anthrax lethal factor cleaves the N-terminus of MAPKKs and induces tyrosine/threonine phosphorylation of MAPKs in cultured macrophages. *Biochem. Biophys. Res. Commun.* 248:706–711.
8. Vitale, G., L. Bernardi, G. Napolitani, M. Mock, and C. Montecucco. 2000. Susceptibility of mitogen-activated protein kinase kinase family members to proteolysis by anthrax lethal factor. *Biochem. J.* 352:739–745.
9. Drum, C.L., S.-Z. Yan, J. Bard, Y.-D. Shen, D. Lu, S. Soelaiman, Z. Grabarek, A. Bohm, and W.-J. Tang. 2002. Structural basis for the activation of anthrax adenylate cyclase exotoxin by calmodulin. *Nature.* 415:396–402.
10. Pellizzari, R., C. Guidi-Rontani, G. Vitale, M. Mock, and C. Montecucco. 1999. Anthrax lethal factor cleaves MKK3 in macrophages and inhibits the LPS/IFNgamma-induced release of NO and TNFalpha. *FEBS Lett.* 462:199–204.
11. Park, J.M., F.R. Greten, Z.W. Li, and M. Karin. 2002. Macrophage apoptosis by anthrax lethal factor through p38 MAP kinase inhibition. *Science.* 297:2048–2051.
12. Agrawal, A., J. Lingappa, S.H. Leppla, S. Agrawal, A. Jabbar, C. Quinn, and B. Pulendran. 2003. Impairment of dendritic cells and adaptive immunity by anthrax lethal toxin. *Nature.* 424:329–334.
13. Dong, C., R.J. Davis, and R.A. Flavell. 2002. MAP kinases in the immune response. *Annu. Rev. Immunol.* 20:55–72.
14. Appleman, L.J., D. Tzachanis, T. Grader-Beck, A.A. van Puijenbroek, and V.A. Boussiotis. 2001. Helper T cell anergy: from biochemistry to cancer pathophysiology and therapeutics. *J. Mol. Med.* 78:673–683.
15. Torgersen, K.M., T. Vang, H. Abrahamsen, S. Yaqub, and K. Tasken. 2002. Molecular mechanisms for protein kinase A-mediated modulation of immune function. *Cell. Signal.* 14:1–9.
16. Cantrell, D. 1996. T cell antigen receptor signal transduction pathways. *Annu. Rev. Immunol.* 14:259–274.
17. Dell'Aica, I., M. Dona, F. Tonello, A. Piris, M. Mock, C. Montecucco, and S. Garbisa. 2004. Potent inhibitors of anthrax lethal factor from green tea. *EMBO Rep.* 5:418–422.
18. Shen, Y., N.L. Zhukovskaya, M.I. Zimmer, S. Soelaiman, P. Bergson, C.R. Wang, C.S. Gibbs, and W.J. Tang. 2004. Selective inhibition of anthrax edema factor by adefovir, a drug for chronic hepatitis B virus infection. *Proc. Natl. Acad. Sci. USA.* 101:3242–3247.
19. Chang, L., and M. Karin. 2001. Mammalian MAP kinase signalling cascades. *Nature.* 410:37–40.
20. Fukuda, M., Y. Gotoh, and E. Nishida. 1997. Interaction of MAP kinase with MAP kinase kinase: its possible role in the control of nucleocytoplasmic transport of MAP kinase. *EMBO J.* 16:901–908.
21. Xia, Y., Z. Wu, B. Su, B. Murray, and M. Karin. 1998. JNKK1 organizes a MAP kinase module through specific and sequential interactions with upstream and downstream components mediated by its amino-terminal extension. *Genes Dev.* 12:3369–3381.
22. Catling, A.D., H.J. Schaeffer, C.W. Reuter, G.R. Reddy, and M.J. Weber. 1995. A proline-rich sequence unique to MEK1 and MEK2 is required for raf binding and regulates MEK function. *Mol. Cell. Biol.* 15:5214–5225.
23. Dhillon, A.S., C. Pollock, H. Steen, P.E. Shaw, H. Mischak, and W. Kolch. 2002. Cyclic AMP-dependent kinase regulates Raf-1 kinase mainly by phosphorylation of serine 259. *Mol. Cell. Biol.* 22:3237–3246.
24. Rao, A., C. Luo, and P.G. Hogan. 1997. Transcription factors of the NFAT family: regulation and function. *Annu. Rev. Immunol.* 15:707–747.
25. Wu, C.-C., S.-C. Hsu, H.-m. Shih, and M.-Z. Lai. 2003. Nuclear factor of activated T cells c is a target of p38 mitogen-activated protein kinase in T cells. *Mol. Cell. Biol.* 23:6442–6454.
26. Baldari, C.T., M.M. Di Somma, M.B. Majolini, C. Ulivieri, E. Milia, and J.L. Telford. 1988. NF-AT-luciferase reporter T cell lines as tools to screen immunosuppressive drugs. *Biologicals.* 26:1–5.
27. Tonello, F., L. Naletto, V. Romanello, F. Dal Molin, and C. Montecucco. 2004. Tyrosine-728 and glutamic acid-735 are essential for the metalloproteolytic activity of the lethal factor of *Bacillus anthracis*. *Biochem. Biophys. Res. Commun.* 313:496–502.
28. Cataldi, A., E. Labruyère, and M. Mock. 1990. Construction and characterization of a protective antigen-deficient *Bacillus anthracis* strain. *Mol. Microbiol.* 4:1111–1117.
29. Boncristiano, M., S. Rossi Paccani, S. Barone, C. Ulivieri, L. Patrussi, D. Ilver, A. Amedei, M.M. D'Elios, J.L. Telford, and C.T. Baldari. 2003. The *Helicobacter pylori* vacuolating toxin inhibits T cell activation by two independent mechanisms. *J. Exp. Med.* 198:1887–1897.
30. Pacini, S., M. Pellegrini, E. Migliaccio, L. Patrussi, C. Ulivieri, A. Ventura, F. Carraro, A. Naldini, L. Lanfrancone, P.G. Pelicci, and C.T. Baldari. 2004. p66SHC promotes apoptosis and antagonizes mitogenic signaling in T cells. *Mol. Cell. Biol.* 24:1747–1757.
31. Plyte, S., M. Boncristiano, E. Fattori, F. Galvagni, S. Rossi Paccani, M.B. Majolini, S. Oliviero, G. Ciliberto, J.L. Telford, and C.T. Baldari. 2001. Identification and characterization of a novel nuclear factor of activated T-cells-1 isoform expressed in mouse brain. *J. Biol. Chem.* 276:14350–14358.