

Lck Dephosphorylation at Tyr-394 and Inhibition of T Cell Antigen Receptor Signaling by *Yersinia* Phosphatase YopH*

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A key virulence factor for *Yersinia pestis*, the etiologic agent of plague, is the tyrosine phosphatase YopH, which the bacterium injects into host cells. We report that treatment of human T lymphocytes with a recombinant membrane-permeable YopH resulted in severe reduction in intracellular tyrosine phosphorylation and inhibition of T cell activation. The primary signal transducer for the T cell antigen receptor, the Lck tyrosine kinase, was specifically precipitated by a substrate-trapping YopH mutant, and Lck was dephosphorylated at its positive regulatory site, Tyr-394, in cells containing active YopH. By turning off Lck, YopH blocks T cell antigen receptor signaling at its very first step, effectively preventing the development of a protective immune response against this lethal bacterium.

Yersinia pestis, the bacterium that causes plague, has in historical times caused devastating pandemics with over 200 million human deaths (1, 2). The classical route of *Y. pestis* infection is from blood-sucking fleas infected from animal reservoirs, mostly rats and other rodents (3). From flea bites, *Yersinia* spreads to local lymph nodes, where the bacterium multiplies rapidly, causing a massive lymphadenitis referred to as bubonic plague within 2 to 6 days (1, 2). Unless treated vigorously during this early stage, the infection progresses into a fatal septicemia and toxemia. A rare and more rapidly fulminant form of infection, pneumonic plague (4), is caused by inhaled bacteria. Although several vaccines exist (5, 6), and *Yersinia* usually is sensitive to streptomycin- and tetracycline-type antibiotics, the pneumonic form of the disease is difficult to treat and still often results in death (7). Because of its rapid replication and effective immune-evading capacity, *Y. pestis* has also been recognized as a potential tool for bioterrorism.

The lethality of *Y. pestis* is conferred by a 70-kb virulence plasmid (8–10), which encodes the Yop (*Yersinia* outer proteins) virulon, a set of proteins that enable extracellular bacteria to disarm cells of the immune system by direct injection of effector proteins (11, 12). A key Yop virulon protein is YopH, a 468-amino acid protein-tyrosine phosphatase (PTPase)¹ (13,

14) with a C-terminal catalytic domain and a multifunctional N-terminal domain, which binds tyrosine-phosphorylated target proteins (15, 16). The catalytic domain of YopH is structurally very similar to that of eukaryotic PTPases (17). Bacterial injection of YopH into phagocytic cell types results in disruption of focal adhesions (18, 19) and inhibition of phagocytosis (20, 21), tumor necrosis factor α release, oxidative burst (22, 23), and hence the inflammatory response to the bacteria. Live bacteria also paralyze T and B lymphocytes (24) and thereby prevent the development of an adaptive immune response. This inhibition depends on YopH (24) and is critical for the survival of the bacteria in the lymph nodes of the infected host, as demonstrated by the protective immunity conferred by several tested vaccines (5, 6). Here we provide a molecular mechanisms for how YopH renders T lymphocytes unresponsive to triggering of their T cell antigen receptor (TCR) and CD28 co-receptor.

MATERIALS AND METHODS

Antibodies and Reagents—The 12CA5 anti-hemagglutinin mAb was from Roche Applied Science. The anti-phosphotyrosine mAb 4G10 was from Upstate Biotechnology, Inc. (Lake Placid, NY). The C305 and OKT3 hybridomas that produce the anti-TCR- β 8 and anti-CD3 ϵ mAbs were from American Type Culture Collection (Manassas, VA). Both mAbs were used as ascites. Anti-CD28 was from Pharmingen. The polyclonal anti-extracellular signal-regulated kinase 2 was from Santa Cruz Biotechnology, Inc. Anti-ZAP-70 was from Zymed Laboratories Inc. (San Francisco, CA). Polyclonal anti-phospho-Erk, anti-phosphoY505-Lck, and anti-PTyr-394-Lck (anti-PTyr-416-Src) were from Cell Signaling Technology Inc. (Beverly, MA).

Plasmids and Site-directed Mutagenesis—DNA encoding YopH was kindly provided by James B. Bliska (Center for Infectious Diseases, SUNY Stony Brook, Stony Brook, NY) and Hans Wolff-Watz (University of Umeå, Umeå, Sweden). The sequence encoding YopH was cloned into pANT, a modified version of pAC28 (25) engineered to add the 16-amino acid sequence of the third α -helix from the *Antennapedia* homeodomain protein (see Fig. 1A) to the N terminus of the insert. The cDNA for green fluorescent protein was also cloned into pANT. YopH was also cloned into the pEGST (25) and into the pEF5HA eukaryotic expression vector, which adds a nine-amino acid hemagglutinin epitope to the N terminus of the insert. The intracellular portion of TCR- ζ in the pGEX prokaryotic expression plasmid was a kind gift from Howard Gray (La Jolla Institute for Allergy and Immunology, San Diego, CA). The expression plasmid for ZAP-70 was described before (26). Site-directed mutagenesis was done using the Transformer™ kit (Clontech, Palo Alto, CA) as recommended by the manufacturer. All mutations were verified by nucleotide sequencing.

Cells and Cell Treatments—Normal T lymphocytes were isolated from venous blood of healthy volunteers by Ficoll gradient centrifugation. Monocytes/macrophages were eliminated by adherence to plastic for 1 h at 37 °C. Jurkat T leukemia cells, and its variants P116, JCaM1, and JCaM2, were kept at logarithmic growth in RPMI 1640 medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, 1 mM sodium pyruvate, nonessential amino acids, and 100 units/ml each of penicillin G and streptomycin. ANT-YopH, ANT-YopH-D356A, and ANT-GFP proteins were induced in transformed *Escherichia coli* and purified using nickel-nitrilotriacetic acid. All three were of equal high

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¹ The abbreviations used are: PTPase, protein-tyrosine phosphatase; mAb, monoclonal antibody; TCR, T cell antigen receptor; GST, glutathione S-transferase; PTyr, phosphotyrosine.

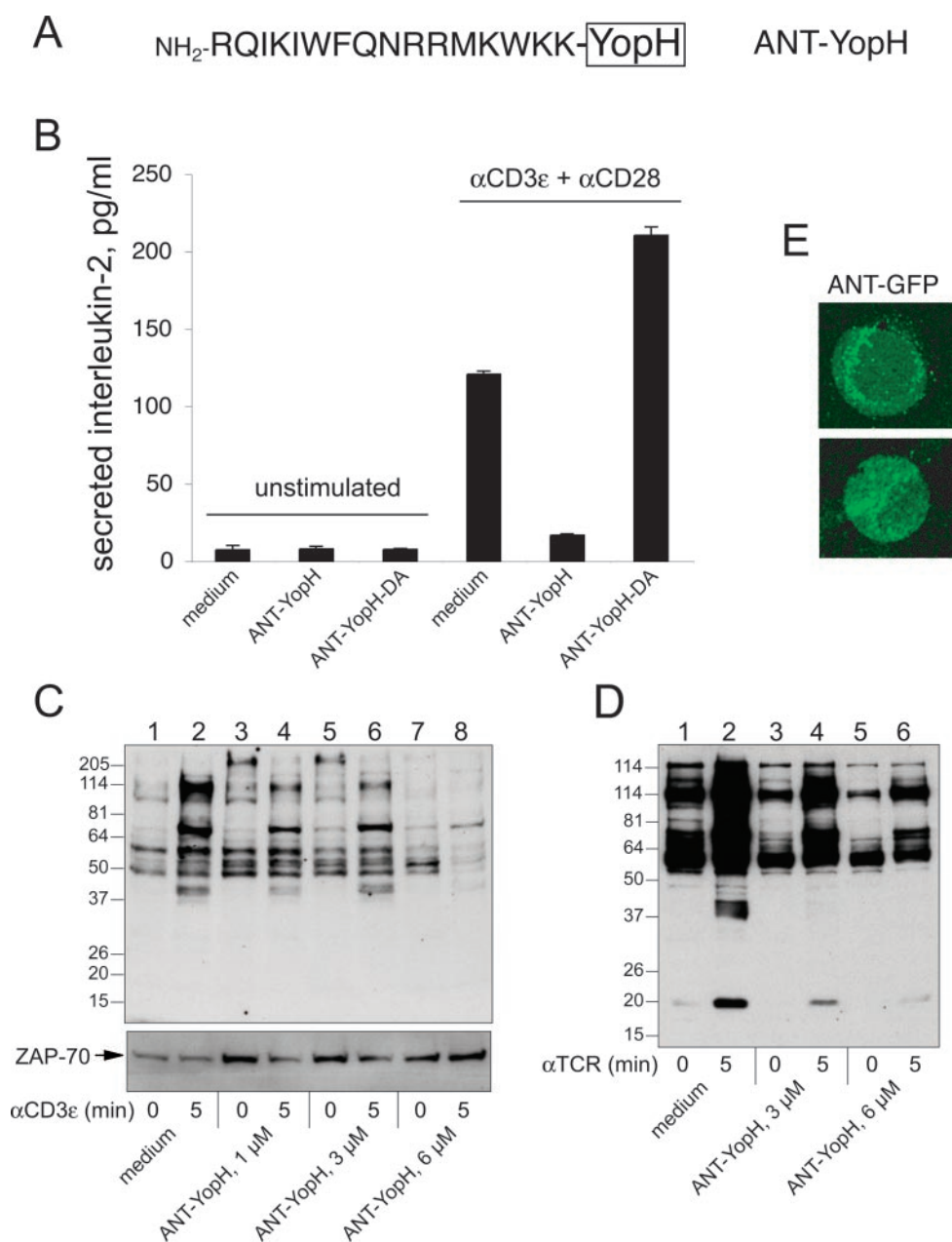


FIG. 1. A membrane-penetrating YopH protein inhibits activation of normal human T lymphocytes and dephosphorylates intracellular proteins on tyrosine. *A*, amino acid sequence of the 16-residue ANTI tag added to the N terminus of YopH. *B*, interleukin-2 secreted into the medium by one million human T lymphocytes treated with medium alone, 6 μ M ANTI-YopH, or 6 μ M catalytically inactive ANTI-YopH-DA for 4 h at 37 $^{\circ}$ C, washed, and then treated with medium alone or with anti-CD3 ϵ plus anti-CD28 mAbs for 20 h, as indicated. *C*, *top panel*, anti-Ptyr immunoblot of lysates of human T lymphocytes treated with medium alone or with 1, 3, or 6 μ M ANTI-YopH and then treated with either medium alone or with anti-CD3 ϵ mAbs for 5 min. *Bottom panel*, loading control blot for ZAP-70 of the same filter. *D*, anti-Ptyr immunoblot of lysates of Jurkat T cells kept in medium alone or in medium with 3 or 6 μ M ANTI-YopH and then treated with either medium alone or with anti-CD3 ϵ mAbs for 5 min. *E*, confocal microscopy of Jurkat cells treated with 6 μ M ANTI-GFP for 5 h.

purity. They were added at 6 μ M (or the indicated lower concentration) to cells in RPMI medium at 37 $^{\circ}$ C. For TCR- and CD28-induced tyrosine phosphorylation responses, normal T lymphocytes were incubated in ice for 15 min with 10 μ g/ml OKT3 and anti-CD28 mAbs, washed, and incubated with a cross-linking sheep anti-mouse Ig for 15 min, washed, and transferred to 37 $^{\circ}$ C for 5 min. Cells were pelleted and lysed in 20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5 mM EDTA containing 1% Nonidet P-40, 1 mM Na₃VO₄, 10 μ g/ml aprotinin and leupeptin, 100 μ g/ml soybean trypsin inhibitor, and 1 mM phenylmethylsulfonyl fluoride and clarified by centrifugation at 15,000 rpm for 20 min. Lysate was mixed with an equal volume of twice concentrated SDS sample buffer, boiled for 1 min, and resolved by SDS-PAGE.

Interleukin-2 Secretion Assay— 5×10^6 human T lymphocytes were treated with 6 μ M ANTI-YopH for 5 h at 37 $^{\circ}$ C in RPMI medium, washed, and stimulated with C305, anti-CD28 mAb, plus a cross-linking anti-mouse Ig for 15 h in 250 μ l of medium with 10% fetal calf serum. 20 μ l

of the supernatant was used for measurement of the amount of interleukin-2 using an enzyme-linked immunosorbent assay kit from Roche Applied Science, as before (27). Results are given as pg/ml of secreted interleukin-2.

Transfections and Luciferase Assays—Transfection of Jurkat T cells and assays for luciferase activity were performed as described previously (28–30) using 10 μ g of pEF5HA-YopH plasmid (or less in Fig. 3) and the dual luciferase system.

Immunoprecipitation, SDS-PAGE, and Immunoblotting—These procedures were done as before (28–30).

Lck In Vitro Kinase Assays—The phosphorylation reaction contained recombinant Lck (Upstate Biotechnology, Inc., Lake Placid, NY) in 25 μ l of 50 mM HEPES, pH 7.5, 150 mM NaCl, 10 mM MnCl₂, 1 mM Na₃VO₄, and 10 μ Ci of [γ -³²P]ATP and 10 μ M ATP and 5 μ g of GST-TCR- ζ as a substrate. After 30 min at 30 $^{\circ}$ C, the reaction was terminated by addition of twice concentrated SDS sample buffer and boiling for 1 min.

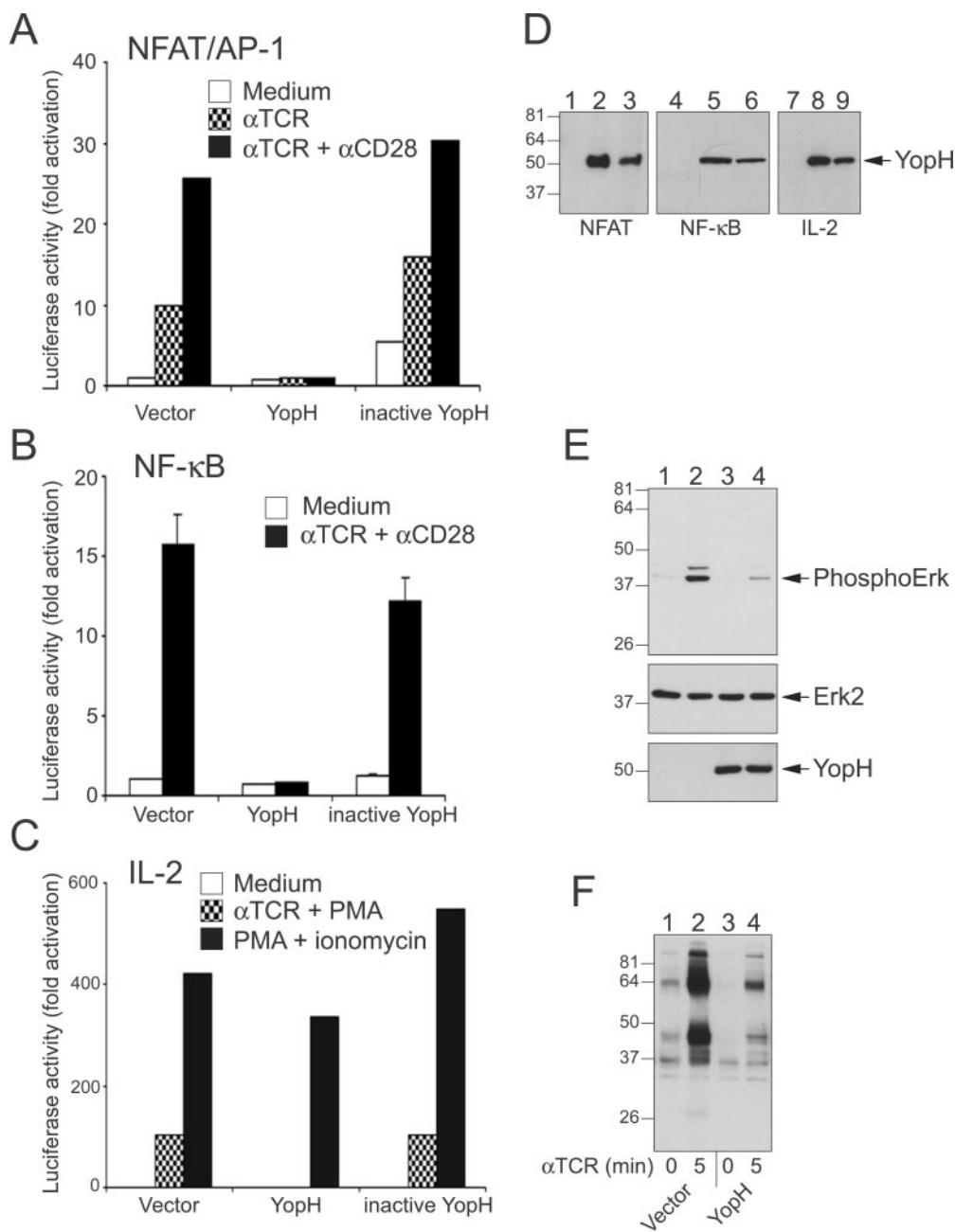


FIG. 2. YopH inhibits TCR signaling pathways. *A*, Jurkat T cells were transiently transfected with a luciferase reporter gene driven by NFAT/AP-1 plus empty vector ($5 \mu\text{g}$) or YopH plasmid ($10 \mu\text{g}$), plus a *Renilla* luciferase plasmid for normalization ($0.3 \mu\text{g}$). Twenty-four h later, the cells were either maintained in RPMI medium alone or stimulated with C305 anti-TCR mAb or anti-TCR plus anti-CD28 mAb, as indicated, for 6 h. Cells were then lysed and assayed for the dual luciferase activity. Firefly luciferase measurements were normalized for *Renilla* luciferase activity, and the data are presented as -fold activation over the unstimulated control sample. *B*, similar experiments with a luciferase reporter gene driven by NF- κ B. *C*, similar experiments using the entire 5' interleukin-2 gene promoter. The cells were stimulated with C305, together with 20 nM phorbol ester, or 0.3 μM ionomycin plus 20 nM phorbol ester, as indicated. *D*, control anti-hemagglutinin epitope tag immunoblots of lysates from the cells used in *A*–*C*. The migration of YopH is indicated. *E*, inhibition of mitogen-activated protein kinase activation by YopH visualized by anti-phospho-Erk immunoblotting of Jurkat T cells transfected with empty pEF5HA vector (lanes 1 and 2) or YopH plasmid (lanes 3 and 4) and either left untreated (lanes 1 and 3) or treated with C305 for 5 min (lanes 2 and 4). *F*, total tyrosine phosphorylation of cellular proteins visualized by anti-phosphotyrosine mAb immunoblotting of Jurkat T cells transfected with empty pEF5HA vector (lanes 1 and 2) or YopH plasmid (lanes 3 and 4) and either left untreated (lanes 1 and 3) or treated with C305 for 5 min (lanes 2 and 4). The DNA encoding YopH was in the pEF5HA eukaryotic expression vector, which adds an N-terminal hemagglutinin epitope tag.

Proteins were resolved on SDS gels and transferred onto nitrocellulose filters for autoradiography.

Substrate-trapping Procedures—For isolation of YopH substrates, 50×10^6 cells were treated with or without 5 $\mu\text{g}/\text{ml}$ of C305 anti-TCR mAb for 5 min, lysed in 20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 2 mM EDTA, and 10 $\mu\text{g}/\text{ml}$ aprotinin and leupeptin. One-third of each sample was precleared with 50 μl of glutathione-Sepharose and then incubated with 5 μg of GST alone or GST-YopH or GST-YopH-D356A. Each protein was precipitated with 20 μl of glutathione-Sepharose, washed extensively in lysis buffer, and analyzed for

bound proteins by SDS-PAGE and anti-PTyr immunoblotting.

Antigen-presenting Cells and Immune Synapses—To visualize antigen-driven TCR signaling, CD8^+ , $\text{OVA}_{257-264}\text{-K}^b$ -specific, OT-I TCR transgenic T cells were mixed with antigen-presenting cells (SigOVA₂₅₇₋₂₆₄MEC-B7.1) for 20 min at 37 °C as before (34), fixed, and stained for phosphotyrosine with the 4G10 anti-PTyr mAb, followed by fluorescein isothiocyanate anti-mouse Ig, with the same protocol as before (30, 31) and an MRC-1024 confocal laser scanning microscope (Bio-Rad). A differential interference contrast image was also taken of each cell.

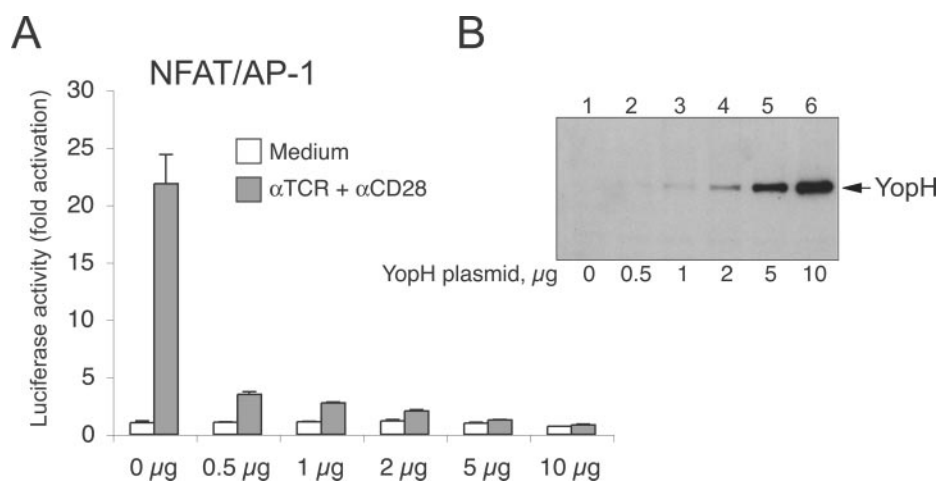


FIG. 3. Dose response for inhibition of reporter gene activation by YopH. *A*, Jurkat T cells were transiently transfected with a luciferase reporter gene driven by NFAT/AP-1 plus empty vector (10 μ g) or YopH plasmid (0–10 μ g, as indicated) plus a *Renilla* luciferase plasmid for normalization (2 μ g). Empty vector was added to obtain the same total amount of DNA for each sample. Twenty-four h later, the cells were either maintained in RPMI medium alone or stimulated with C305 anti-TCR mAb plus anti-CD28 mAb for 6 h. Cells were then lysed and assayed for the dual luciferase activity. Firefly luciferase measurements were normalized for *Renilla* luciferase activity, and the data are presented as -fold activation over the unstimulated control sample. *B*, anti-hemagglutinin tag immunoblot of lysates from the same transfectants. Note that YopH inhibited activation of the reporter gene quite well at the lowest concentration of DNA plasmid even when the protein is barely detectable.

RESULTS

Membrane-permeable YopH Inhibits Activation of Normal T Lymphocytes—To mimic the scenario in a lymph node of an infected patient, where the bacteria inject YopH protein directly into the cytoplasm of normal untransformed T lymphocytes, we constructed a membrane-permeable YopH protein by fusing the 16-amino acid residue third α -helix from the *Antennapedia* homeodomain protein (32) (Fig. 1A) to the N terminus of YopH and then treated normal human T lymphocytes with the resulting fusion protein (ANT-YopH) or its catalytically inactive point-mutant for 4 h at 37 $^{\circ}$ C. Treated cells were washed and stimulated through their TCR and CD28 co-receptor for 20 h, followed by measurement of interleukin-2 released into the medium. Although stimulated control cells produced 120.5 ± 2.2 pg/ml of interleukin-2 (compared with 7.1 ± 2.8 ng/ml from non-stimulated cells), the stimulated ANT-YopH-treated cells produced only 16.5 ± 0.9 ng/ml of interleukin-2 (compared with 7.8 ± 1.6 pg/ml from the non-stimulated cells), a 92.3% reduction in response (Fig. 1B). Very similar results were obtained in a second independent experiment. In contrast, T lymphocytes treated with a catalytically inactive ANT-YopH protein produced 210.4 ± 5.3 pg/ml of interleukin-2 (a 74% increase), demonstrating that YopH must be an active PTPase to block T cell activation. Indeed, the active ANT-YopH reduced the levels of tyrosine phosphorylation in normal T lymphocytes (Fig. 1C), as well as in Jurkat T leukemia cells (Fig. 1D), and prevented the normal TCR-induced response. These effects were dose- and time-dependent and did not compromise cell viability. Intracellular delivery was also confirmed using green fluorescent protein with the 16-amino acid *Antennapedia* tag (ANT-GFP; see Fig. 1E), and no effects were observed by YopH lacking the 16-amino acid ANT tag (not shown).

Inhibition of T Cell Activation by Transfected YopH—To study the molecular basis for inhibition of T cell activation by YopH, we transfected Jurkat T cells with an expression plasmid encoding YopH with an N-terminal hemagglutinin epitope tag. The cells were stimulated with anti-TCR plus anti-CD28 mAbs, and the activation of co-transfected luciferase reporter genes was measured. Expression of YopH completely blocked any TCR-induced activation of reporters driven by NFAT/AP-1 (Fig. 2A) or NF- κ B (Fig. 2B) or by the entire 5' promoter of the interleukin-2 gene (Fig. 2C). Only phorbol ester plus calcium ionophore, which bypass all receptor-proximal tyrosine phos-

phorylation events, induced a 80% interleukin-2 response in YopH-expressing cells (Fig. 2C). Catalytically inactive YopH did not affect T cell activation (Fig. 2, A–C), and the effect of YopH was dose-dependent with near-maximal inhibition seen at the lowest detectable levels of YopH (Fig. 3). Activation of the Erk mitogen-activated protein kinase was also inhibited by YopH (Fig. 2E), as was TCR-induced tyrosine phosphorylation of cellular proteins (Fig. 2F). Together, these results confirm that YopH is a very potent inhibitor of TCR signaling and that its inhibitory effect depends on its PTPase activity.

Lck Is a Key Substrate for YopH in T Cells—To determine which T cell protein(s) YopH dephosphorylates to block TCR signaling, we created GST fusion proteins of YopH and of its substrate-trapping (33) D356A mutant. These proteins were incubated with lysates of resting or activated T cells, washed, and analyzed for bound tyrosine-phosphorylated proteins by immunoblotting (Fig. 4A). The substrate-trapping mutant clearly bound more proteins than the non-mutated active YopH, suggesting that YopH can interact with T cell phosphoproteins through both its N-terminal domain and its C-terminal catalytic domain. Several of these proteins were identified by immunoblotting with antibodies against known TCR signaling proteins (34), such as Vav, LAT, ZAP-70, and Lck. Because GST control samples did not contain any of these proteins, their presence in the YopH and YopH-D356A samples is likely the result of direct or indirect binding to the N-terminal targeting domain or the C-terminal catalytic domain of YopH. Importantly, Lck was detected only in samples obtained with the substrate-trapping YopH-D356A mutant (Fig. 4A, bottom panel). Because this kinase is the first PTK to be activated by the TCR (34), we decided to examine whether YopH has direct effects on this kinase. First, we immunoprecipitated Lck with anti-phosphotyrosine mAbs from resting or TCR-triggered cells transfected with empty vector or YopH and found that YopH caused a dramatic reduction in Lck present in these immunoprecipitates (not shown). To further substantiate this result, we immunoblotted cell lysates with phospho-specific antibodies that selectively recognize the two principal tyrosine phosphorylation sites in Lck. The positive regulatory phosphorylation site (35) Tyr-394 was found to be dephosphorylated by YopH (Fig. 4B, top panel), particularly following TCR triggering (lane 4), whereas the negative regulatory site (35) Tyr-505 was marginally dephosphorylated in YopH-expressing T cells (Fig. 4B,

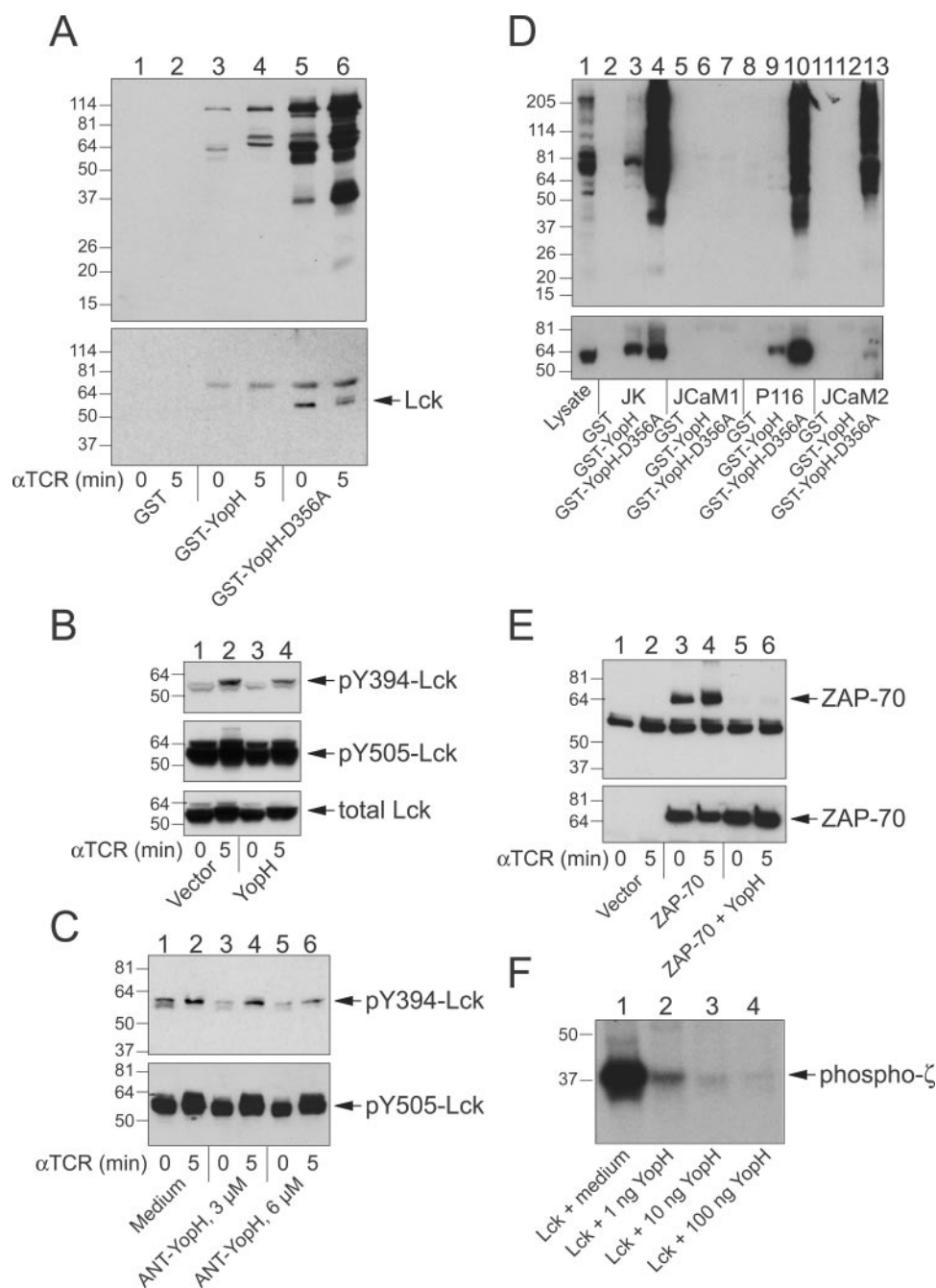


FIG. 4. The positive regulatory tyrosine 394 of the Lck kinase is a key substrate for YopH. *A*, top panel, anti-phosphotyrosine immunoblot of proteins bound to GST (lanes 1 and 2), GST-YopH (lanes 3 and 4), or the substrate-trapping mutant GST-YopH-D356A (lanes 5 and 6), from Jurkat T cells treated without (lanes 1, 3, and 5) or with C305 for 5 min (lanes 2, 4, and 6). Bottom panel, anti-Lck immunoblot of the same filter. The ~75-kDa band is the GST fusion protein. *B*, top panel, anti-PTyr-394-Lck immunoblot of lysates of Jurkat T cells transfected with empty vector (lanes 1 and 2) or YopH (lanes 3 and 4) and either left untreated (lanes 1 and 3) or treated with C305 for 5 min (lanes 2 and 4). Middle panel, anti-PTyr-505-Lck immunoblot of the same samples. Bottom panel, anti-Lck blot of the same samples to show that Lck levels did not change. Also note the molecular weight shift in Lck upon TCR triggering. *C*, top panel, anti-PTyr-394-Lck immunoblot of lysates of T cells preincubated with medium alone (lanes 1 and 2) or with 3 μM (lanes 3 and 4) or 6 μM ANT-YopH and either left untreated (lanes 1, 3, and 5) or treated with C305 for 5 min (lanes 2, 4, and 6). Bottom panel, anti-PTyr-505-Lck immunoblot of the same filter. *D*, top panel, anti-phosphotyrosine immunoblot of proteins bound to GST (lanes 2, 5, 8, and 11), GST-YopH (lanes 3, 6, 9, and 12), or the substrate-trapping mutant GST-YopH-D356A (lanes 4, 7, 10, and 13), from the indicated sublines of Jurkat treated with 100 μM pervanadate for 3 min. JK, parental Jurkat; JCaM1, lacking Lck; P116, lacking ZAP-70; JCaM2, lacking LAT. Lane 1 is a cell lysates from parental Jurkat. Bottom panel, anti-Lck immunoblot of the same filter. *E*, top panel, anti-PTyr immunoblot to show TCR-induced tyrosine phosphorylation of ZAP-70 in Jurkat T cells transfected with empty pEF5HA vector, ZAP-70, or ZAP-70 plus YopH. Bottom panel, anti-ZAP-70 immunoblot of the same filter. *F*, autoradiogram of an *in vitro* kinase reaction of recombinant Lck with GST-TCR-ζ as a substrate. Prior to the assay, Lck was preincubated with buffer alone (lane 1) or with 1 (lane 2), 10 (lane 3), or 100 ng (lane 4) of GST-YopH for 30 min at 37 °C. Subsequent action of YopH was prevented by addition of 5 mM Na₃VO₄.

middle panel). The amount of Lck was not altered by YopH (Fig. 4B, bottom panel). Addition of ANT-YopH to the cells similarly reduced Tyr-394 phosphorylation of Lck (Fig. 4C, top panel) but not Tyr-505 phosphorylation (Fig. 4C, bottom panel).

Because Tyr-394 phosphorylation is a prerequisite for catalytic activity of Lck toward its cellular substrates (35), we tested whether other phosphoproteins precipitated by the substrate-trapping YopH-D356A also depended on Lck. Although multi-

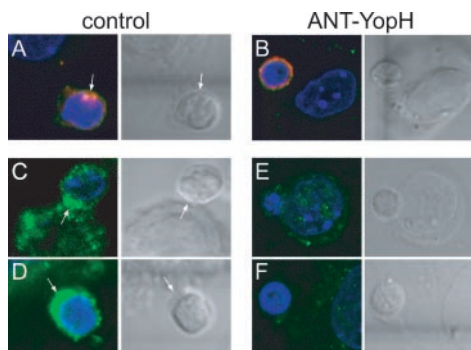


FIG. 5. YopH prevents the formation of an immune synapse with Lck recruitment and tyrosine phosphorylation. *A* and *B*, confocal images of antigen-specific T cells pretreated with medium alone (*A*) or with 6 μ M ANT-YopH (*B*) and then incubated with antigen-presenting cells for 20 min. The cells were stained for Lck (green), CD3 ϵ (red), and DNA (blue). The immune synapse is indicated with a white arrow. *B–F*, confocal images of antigen-specific T cells pretreated with medium alone (*C* and *D*) or with 6 μ M ANT-YopH (*E* and *F*) and then incubated with antigen-presenting cells for 20 min. The cells were stained for phosphotyrosine (green) and DNA (blue). The immune synapse is indicated with a white arrow. The images on the right are Nomarski contrast images of the same cells. The shown cells are representative of the majority of cells.

ple phosphoproteins were captured from Jurkat cells, none were detected in samples from the Lck-negative JCaM1 subline of Jurkat (Fig. 4*D*, top panel) even after pervanadate treatment to maximize tyrosine phosphorylation. In contrast, numerous proteins precipitated from the ZAP-70-negative P116 and LAT-deficient JCaM2 sublines. These results suggest that Lck is necessary for any protein to bind the substrate-trapping YopH. In further agreement with the notion that YopH targets Lck, the Lck-mediated phosphorylation of ZAP-70 was blocked by YopH (Fig. 4*E*). Finally, by dephosphorylating Tyr-394, YopH suppressed the catalytic activity of recombinant Lck *in vitro* (Fig. 4*F*).

YopH Prevents the Formation of an Immune Synapse—Because phosphate at Tyr-394 is absolutely necessary for substrate phosphorylation by Lck, YopH will effectively turn off Lck and thereby block TCR signaling at its most receptor-proximal step. To address this notion under more physiological conditions, we treated antigen-specific OT-I TCR transgenic T cells with ANT-YopH and then mixed the cells with antigen-presenting cells (SigOVA_{257–264}/MEC-B7.1) for 20 min at 37 °C. The cells were then fixed, stained with antibodies, and viewed under a confocal microscope. Although untreated cells formed immune synapses with the antigen-presenting cells, as visualized by round contact structures containing CD3 and Lck (Fig. 5*A*) and enriched in tyrosine-phosphorylated proteins (Fig. 5, *C* and *D*), ANT-YopH-treated cells were completely unable to form immune synapses (Fig. 5*B*) and remained negative for anti-PTyr (Fig. 5, *E* and *F*). In essence, YopH rendered the T cells completely unresponsive to the presence of antigen.

DISCUSSION

Pathogenic bacteria have evolved numerous ways of evading the innate and adaptive immune systems. One of the most successful strategies was adopted by *Y. pestis*, namely a type III secretion system that injects a set of tranquilizing proteins directly into the cytoplasm of macrophages and lymphocytes that the bacterium encounters in the lymph nodes of infected individuals. As a result, the targeted cells become unable to respond, and the bacteria can multiply unopposed by the normal mechanisms of host defense.

We show that one of the injected effectors, the YopH PTPase, functions in T lymphocytes by dephosphorylating Lck at Tyr-394, resulting in a complete loss of Lck activity and, therefore,

any tyrosine phosphorylation of downstream signaling proteins or formation of an immune synapse enriched in tyrosine-phosphorylated signaling complexes. By this mechanism, YopH will completely paralyze T cells and thereby prevent the development of an adaptive immune response.

Lck is upstream of nearly all tyrosine phosphorylation events in TCR signaling, as illustrated by the very low levels of phosphotyrosine in the Lck-negative JCaM1 cell line. Thus, the inactivation of Lck by YopH results in loss of phosphorylation of all downstream targets, a situation that is virtually indistinguishable from a broad and indiscriminate dephosphorylation of proteins by YopH. Indeed, it is likely that YopH does dephosphorylate a few other cellular proteins (e.g. other Src family kinases). There is no conceivable reason for a bacterial PTPase to maintain absolute monospecificity; as long as key substrates like Lck are dephosphorylated, it does not harm the bacteria if other proteins are dephosphorylated as well. However, the targeting of Lck Tyr-394 is a much more efficient way to block TCR signaling than an indiscriminate dephosphorylation of multiple proteins, because the dephosphorylation of relatively few Lck molecules will have a substantial impact. This is particularly important for live bacteria in the lymph nodes of infected patients, where each bacterium encounters numerous host cells and can only inject a limited amount of YopH into each T cell. Because most phosphotyrosine-containing proteins are continuously dephosphorylated by efficient endogenous PTPases, a sharp decline in their rate of phosphorylation following Lck inactivation will result in a rapid clearance of global tyrosine phosphorylation. In contrast, a contribution by YopH to the already rapid rate of dephosphorylation of most targets of Lck or downstream kinases would have a much smaller effect.

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