Human Cytolytic T Cell Recognition of *Yersinia pestis* Virulence Proteins That Target Innate Immune Responses

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Cell contact by the plague bacterium *Yersinia pestis* initiates the injection of several virulence factors that target biochemical pathways critical for host clearance of bacteria. Despite this impairment of innate immunity, it is unclear whether antigen recognition by T cells is equally affected. We present evidence that human cytolytic T cells respond to *Y. pestis* virulence proteins presented by infected monocytes and dendritic cells. These T cell antigens consisted of a panel of proteins encoded by pCD1, a 70-kDa plasmid that harbors virulence factors and transport proteins of the cell contact–dependent, type III secretion system. Infected cells retained the ability to process and present tetanus toxoid to T cells, which indicates that responses to unrelated antigens were also maintained. Our results indicate that T cell immunity remains functional during *Y. pestis* infection, which thus suggests the potential benefits of therapeutic vaccination and strategies that emphasize the inclusion of cytotoxic T lymphocyte responses.

The isolation of multidrug-resistant strains of *Yersinia pestis* during recent disease outbreaks [1] and the potential risk of bioterrorism [2, 3] have renewed concern for plague as a public-health issue. Monocytes, macrophages, and other phagocytes are the first cellular components of innate immunity to encounter *Y. pestis* bacilli immediately after injection by fleas. If they are disseminated from draining lymph nodes to the lungs, bacteria can be transmitted from person to person in aerosols generated by sneezing or coughing, which potentially leads to outbreaks of primary pneumonic plague [4, 5]. A cell contact–dependent type III secretion system (TTSS) injects *Yersinia* outer proteins (Yops) and additional virulence factors into the cytosol, which impairs several biochemical pathways associated with innate immunity. Translocation of the TTSS-associated factors into host cells results in profound changes in cytoskeletal dynamics, signal transduction, and cell-cell communication and ultimately progresses to apoptotic cell death [6, 7]. Phagocytic cell death may allow bacteria to proliferate extracellularly [8] and to establish sequelae linked to morbidity and lethality. Furthermore, *Yersinia* species can survive and replicate within macrophages for a limited time [9], perhaps during the earliest phase of disease. Thus, the potential contribution of T cells to host responses during the brief intracellular phase of plague is an unresolved issue.

Vaccines against *Y. pestis* infection have primarily focused on protective antibody responses to the fraction 1 capsular (CaF1) and low calcium response V (LcrV) proteins [10–13]. CaF1 is an antiphagocytic factor [14], and LcrV is a regulator of Yop production and translocation into host cells [15, 16] that may also directly contribute to virulence [17]. In addition to the reported role played by neutralizing antibody in immune protec-

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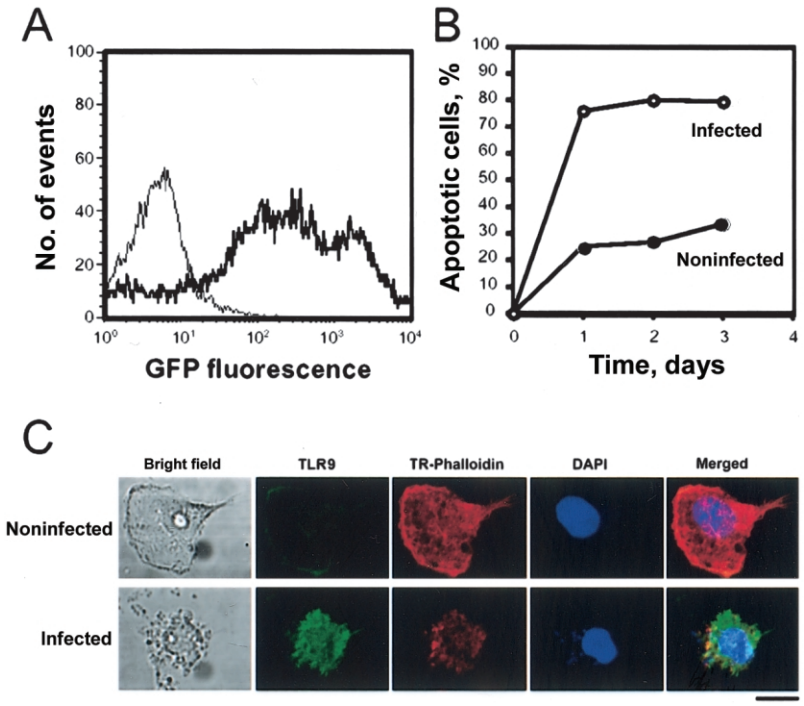

Figure 1. *A,* Histogram of *Yersinia pestis* infection of monocytes ($CD14^+$ cells) by green fluorescent protein (GFP)–transformed *Y. pestis (thick line),* relative to uninfected monocytes *(thin line). B,* Induction of apoptosis by bacterial infection. Freshly isolated monocytes were infected with *Y. pestis,* permeabilized, and labeled with phycoerythrin-conjugated anti–caspase 3 (Apo 2.7) or isotype-matched control antibody. Bound antibody was measured by flow cytometry. *C,* Disruption of focal adhesions, cell rounding, and apoptosis caused by monocyte infection with *Y. pestis.* Adhered monocytes were infected with *Y. pestis,* fixed, and made permeable for antibody labeling. Actin microfilaments were detected with Texas red (TR)–labeled phalloidin, Toll-like receptor (TLR)–9 with specific monoclonal antibody, followed by fluorescein isothiocyanate–conjugated rabbit anti–mouse IgG, and nuclei with 4′,6- diamidino-2-phenylindole (DAPI). Bar, 10 μm.

tion [18, 19], cytotoxic T lymphocyte (CTL) responses to F1 have been described in mice [20]. Immunity to infection by the closely related enteropathogenic bacteria *Y. enterocolitica* [21, 22] and *Y. pseudotuberculosis* [23] is mediated by $CD8^+$ T cells in mice. Furthermore, lysis by major histocompatibility complex class I–restricted $CD8^+$ CTLs required the sensitization of lymphoblast target cells by extracellular adherence of *Y. pseudotuberculosis* to the target cell surface by the bacterial ligand invasin [23]. However, *Y. pestis* does not express invasin because of disruption of the gene by an insertional element [24], which suggests that host responses might also differ in some respects from the enteropathogenic species of *Yersinia.* A recent publication suggested that *Y. pestis* disables antigen-presenting cells (APCs) and host immune responses during plague [25]. On the basis of the hypothesis that primary CTL responses might be critical for immunity, we examined mononuclear cells obtained from healthy donors with no apparent history of plague, to determine whether these primary T cell responses were impaired by the active infection of APCs. T cell responses to infected monocytes and dendritic cells (DCs) were studied using a panel of recombinant TTSS proteins encoded by pCD1, a large virulence plasmid that contains 96 open reading frames that play a pivotal role in controlling innate immunity. Although TTSS-associated proteins represent a small fraction of the >4300 total proteins produced by *Y. pestis,* these may be the most important for CTL-mediated immunity.

MATERIALS AND METHODS

Reagents and antibodies. Pooled human AB serum was obtained from Pel-Freez. Mouse anti-human CD14 and CD3 monoclonal antibodies (MAbs) conjugated with magnetic beads were purchased from Miltenyi Biotech. The fluorescein isothiocyanate (FITC)–conjugated MAb Leu M3 (anti-CD14) and immunoglobulin isotype control antibodies were purchased from Becton Dickinson. FITC-labeled goat anti–mouse IgG was purchased from Zymed. MAb to 7A6 antigen (phycoerythrin [PE]–labeled Apo 2.7 antibody, 2.7A6A3 clone) was purchased from Immunotech. Interleukin (IL)–15 was purchased from Pepro Tech. Ficoll-hypaque was purchased from Pharmacia. The luciferase-based Vialight cytotoxicity assay kit was purchased from Lumitech. The [methyl-^{3}H]thymidine was purchased from Amersham Life Sciences. The cytometric bead array (CBA) human inflammation kit was purchased from BD

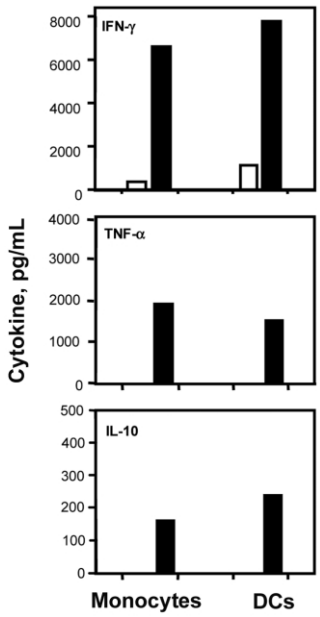

Figure 2. T cell activation by intracellular *Yersinia pestis*. Monocytes or dendritic cells (DCs) were infected with *Y. pestis* (10 bacteria/cell) and cultured with or without autologous T cells. Uninfected monocytes were also cultured with autologous T cells as controls. Culture supernatants were collected after 24 h of culture, and cytokine levels were measured. White bar, T^- cells; black bar, T^+ cells. IFN, interferon; IL, interleukin; TNF, tumor necrosis factor.

Biosciences Pharmingen. The carboxyl-terminal peptide (451 aa residues) of tetanus toxin (C fragment) was purchased from Roche Applied Science. Anti–Toll-like receptor (TLR)–9 antibody was obtained from e-Biosciences. Vectashield mounting medium that contained 4′,6-diamidino-2-phenylindole was purchased from Vector Laboratories. Texas red–phalloidin was purchased from Molecular Probes.

Cell isolations and cultures. Peripheral blood mononuclear cells were obtained from consenting healthy donors, in accordance with guidelines of the institutional review board–approved research donor protocol, and were isolated by standard density-gradient centrifugation at 400 *g* with Ficoll-hypaque (Pharmacia). Monocytes ($CD14^+$) were isolated as described elsewhere [26]. $CD14^+$ monocytes were incubated for 48 h with recombinant IL-15 (100 ng/mL) to prepare DCs, as described elsewhere [26]. Cell purities were >98% for the isolated mononuclear-cell subsets used in all reported experiments.

Infection of monocytes and T cell activation. The *Y. pestis* strain CO92 (*pgm*$^-$, *pla*$^-$) has been described elsewhere [27]. Bacteria were cultured (for 12 h at 37°C) from a single colony in heart-infusion broth (Difco Laboratories) supplemented with 0.2% xylose and 2.5 mmol/L $CaCl_2$. A primed TTSS was confirmed by Western blotting of bacterial lysates and Yop-specific rabbit antibodies (R.G.U., data not shown). Bacteria were washed 3 times by centrifugation at 1600 *g* with RPMI 1640 medium before being used in experiments. For use in selected studies, *Y. pestis* CO92 (*pgm*$^-$, *pla*$^-$) was transformed with pGFPuv (Invitrogen), using standard electroporation techniques, to facilitate the expression of green fluorescent protein (Yp-GFP). Monocytes were incubated (for 1 h at 37°C) with Yp-GFP, washed, and analyzed for the internalization of bacteria by flow cytometry. For measuring T cell activation, freshly isolated monocytes were infected (for 1 h) with *Y. pestis* (10 bacteria/cell), extracellular bacteria were removed, and gentamicin (10 μg/mL) was added before culturing (6 days) with autologous T cells. The T cells were isolated using paramagnetic beads coated with anti-CD3 MAbs (Miltenyi Biotech), as described elsewhere [28]. Activated cells were labeled with PE-conjugated anti-CD69 antibody, FITC–conjugated anti-CD4 or anti-CD8, or isotype-matched control antibody and analyzed by flow cytometry.

Cell markers of infection and TTSS activity. Freshly isolated monocytes were infected with *Y. pestis* (10 bacteria/cell), harvested at different time points, then permeabilized and labeled with PE-conjugated anti–caspase 3 antibody (Apo 2.7) or isotype-matched control antibody. Apo 2.7-PE antibody reacts with the mitochondrial membrane protein (7A6 antigen) when it is undergoing apoptosis [29, 30]. Antibody labeling was measured by flow cytometry. To measure focal adhesions, monocytes were layered on coverslips and allowed to adhere for 2 h. Cells were then incubated with *Y. pestis* (for 45 min at 37°C), washed, and cultured for an additional 20 min with antibiotic (RPMI 1640, 5% human AB serum, and 10 μg/μL gentamicin). The coverslips were gently washed, to remove free bacteria, and were incubated for an additional 24 h. Cells were fixed and permeabilized before analysis by confocal microscopy.

Expression and purification of* Y. pestis *proteins. Recombinant CaF1 was expressed in *Escherichia coli* and purified as described elsewhere [10]. The recombinant proteins YopH, YopM, YopK, YopE, *Yersinia* protein kinase (Ypk)–A, LcrG, LcrH, *Yersinia* secretion protein (Ysc)–M1, *Yersinia* modulator

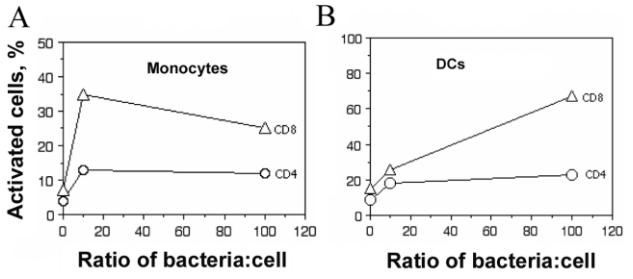

Figure 3. *Yersinia pestis*–infected monocytes and dendritic cells (DCs) and enhanced the activation of $CD8^+$ T cells. Uninfected or *Y. pestis*–infected monocytes or DCs (10 or 100 bacteria/cell) were cultured with autologous T cells (1×10^7 antigen-presenting cells:5×10^7 T cells). Cells were harvested after 6 days, expression of CD69 among $CD4^+$ and $CD8^+$ T cells was measured by dual-color flow cytometry.

Table 1. Recombinant *Yersinia pestis* antigens examined.

Protein	Amino acid sequence
LcrG	1–95
LcrH	1–168
SycE	1–130
SycH	1–138
TyeA	2–81
YmoA	1–67
YopH	2–129
YopD	1–308
YopD	150–308
YopH	164–468[a]
YopK	1–182
YopM	1–526[b]
YopR	2–165
YscE	2–66
YscM1	1–114
YscM1	29–78
YscM2	1–116[c]
YscP	1–115
CaF1	1–170

NOTE. CaF, fraction 1 capsular protein; Lcr, low calcium response; Syc, specific Yop chaperone; Tye, translocation of Yops into eukaryotic cells; Ymo, *Yersinia* modulator; Yop, *Yersinia* outer protein; Ysc, *Yersinia* secretion protein.

[a] Includes the mutation D356A.
[b] Cloned from *Y. enterocolitica*.
[c] YscM1 homolog from *Y. enterocolitica* not expressed by *Y. pestis*.

(Ymo)–A, YopR, translocation of Yops into eukaryotic cells (Tye)–A, YscP, and YscE were produced and characterized as described elsewhere [31]. The coding sequence for YopD was amplified from *Y. pestis* CO92 genomic DNA by polymerase chain reaction that used PfuTurbo (Stratagene) DNA polymerase and was cloned into the vector pDONR201 (Invitrogen). Gene inserts confirmed by DNA sequencing were cloned by recombination into the *E. coli* expression vector pDEST17 (Invitrogen), and plasmids were transformed into *E. coli* BL21 host cells (Novagen) for protein expression. All of the recombinant *Yersinia* proteins were expressed in *E. coli* BL21 (DE3) cells and purified to homogeneity using a combination of amylose-affinity chromatography and/or immobilized metal-affinity chromatography and conventional chromatographic techniques. All proteins were dialyzed in RPMI 1640 before use, and low endotoxin levels were confirmed using the limulus lysate assay. The molecular weights of the final products were confirmed by electrospray mass spectrometry, and purity and identity were confirmed by SDS-PAGE.

T cell proliferation assay. For the detection of T cell proliferative responses to *Yersinia* antigens, $CD14^+$ monocytes from HLA-A2 donors were infected with *Y. pestis* at the start of culture (10 bacteria/cell). Gentamicin or tetracycline (10 μg/mL) was added to cultures after 2 h of infection. Monocytes were washed extensively to remove extracellular bacteria before autologous T cells were added to the culture at 24 h. Primary T cell cultures were then collected after 6 days of incubation, and $CD3^+$ T cells were purified using anti-CD3 antibody conjugated to magnetic beads as described elsewhere [32]. After 6 days of culture with purified recombinant *Yersinia* antigens presented by fresh DCs (prepared as described above), cultures were pulsed with 1 μCi of [^{3}H]thymidine/well for 10 h, and proliferation was measured by harvesting the cells onto microplate unifilters and measuring radioactivity in a liquid scintillation counter (Packard).

Cytotoxicity assay. Cytotoxicity assays were performed as described elsewhere [32]. Briefly, monocytes were obtained from a consenting HLA-A2 donor. To transform monocytes to DCs, monocytes were cultured with IL-15 for 48 h, as described elsewhere [26]. Adherent cells were washed extensively, and autologous T cells were added to the culture with antigens (10 μg/mL) and incubated for 6 days. T cells were collected and used as effector cells in cytotoxicity assays. Homozygous B lymphoblasts (HLA-A2; BSM cells) were pulsed (for 1 h at 37°C) with *Y. pestis* proteins (10 μg/mL) and used as target cells (5×10^3/well) for specific effector T cells. Cultures were incubated (100 μL for 4 h), then lysed with 100 μL of a nucleotide-releasing agent (Lumi tech), followed by the addition of an ATP-monitoring agent (20 μL). Light emission was measured in a luminometer (Wallac 1420 Victor; Perkin Elmer), and specific cytotoxicity was calculated by subtracting experimental from maximum target-cell lysis.

Cytokine analysis. Cytokines in culture supernatants were measured with a CBA kit (BD Biosciences Pharmingen) using capture beads coated with antibodies specific for cytokines and flow-cytometric analysis, in accordance with the manufacturer's instructions, as described elsewhere [28].

RESULTS

***T cell recognition of monocytes and DCs infected with* Y. pestis.** Normal human $CD14^+$ monocytes were incubated with *Y. pestis* in culture to confirm that the TTSS was functional with these cells. Approximately 70%–80% of monocytes were consistently observed to be infected (figure 1*A*), as measured by monitoring the internalization of *Y. pestis* that stably expressed GFP. In confirmation of previous observations [28], we noted elevated cytoplasmic levels of TLR9, an innate immune receptor of bacterial DNA. Expression of the mitochondrial membrane protein caspase 3 (7A6 antigen), a marker of the early stage of apoptosis [29, 30], was greatly increased in infected cells, compared with uninfected controls (figure 1*B*), and was also an anticipated effect of Yops delivered by the TTSS.

Disruption of focal adhesions, cell rounding, and apoptosis were evident, which is consistent with a perturbation of biochemical networks by bacterial infection (figure 1*C*). In addition, viable replicating bacteria were recovered from the monocytes 48 h after infection (data not shown). These observations confirmed that innate immune functions of the infected monocytes were likely impaired. We next cultured *Y. pestis*–infected monocytes and DCs with autologous T cells for 24 h and examined culture supernatants for levels of inflammatory cytokines (figure 2). We anticipated that the capacity of both cell types to activate primary T cell responses should converge by the end of culture, because previous studies demonstrated that monocytes infected with *Y. pestis* differentiated into mature DCs [28]. Increased levels of tumor necrosis factor–α and interferon-γ were measured in the supernatants from cultures of both infected monocytes and myeloid-derived DCs cocultured with T cells, compared with control cultures of infected monocytes or DCs without T cells. Culture supernatants from T cells with uninfected monocytes exhibited negligible amounts of cytokines (data not shown). The majority of T cells activated by infected monocytes or DCs were $CD8^+$ (figure 3*A* and 3*B*), which suggests that cytolytic T cell responses may dominate. Collectively, these results demonstrated that infected monocytes and DCs were both capable of activating primary T cell responses to *Y. pestis*.

***T cell responses to TTSS proteins of* Y. pestis.** We next examined T cell recognition of recombinant *Y. pestis* proteins by T cells primed with infected monocytes or DCs. A subset of TTSS proteins encoded by *Y. pestis* pCD1 was prepared as full-length proteins or structural domains, (table 1), as described elsewhere [31]. A recombinant capsular CaF1 protein, an intensively studied protective antigen, was also used as a positive control. Autologous T cells were cocultured with *Y. pestis*–infected monocytes or DCs, and these in vitro–primed T cell cultures were then tested for recognition of secondary responses to TTSS proteins. We measured CTL responses from HLA-A2 donors using BSM target cells pulsed with individual proteins from the recombinant panel. When infected monocytes were used to prime T cell responses (figure 4, *top*), significant cytolysis was observed for the following proteins: $YopH_{2–129}$, YopK, YopM, TyeA, YscM2, YscE, specific Yop chaperone (Syc)–E, LcrG, YscP, and LcrH. CTL recognition of CaF1, the protective antigen de-

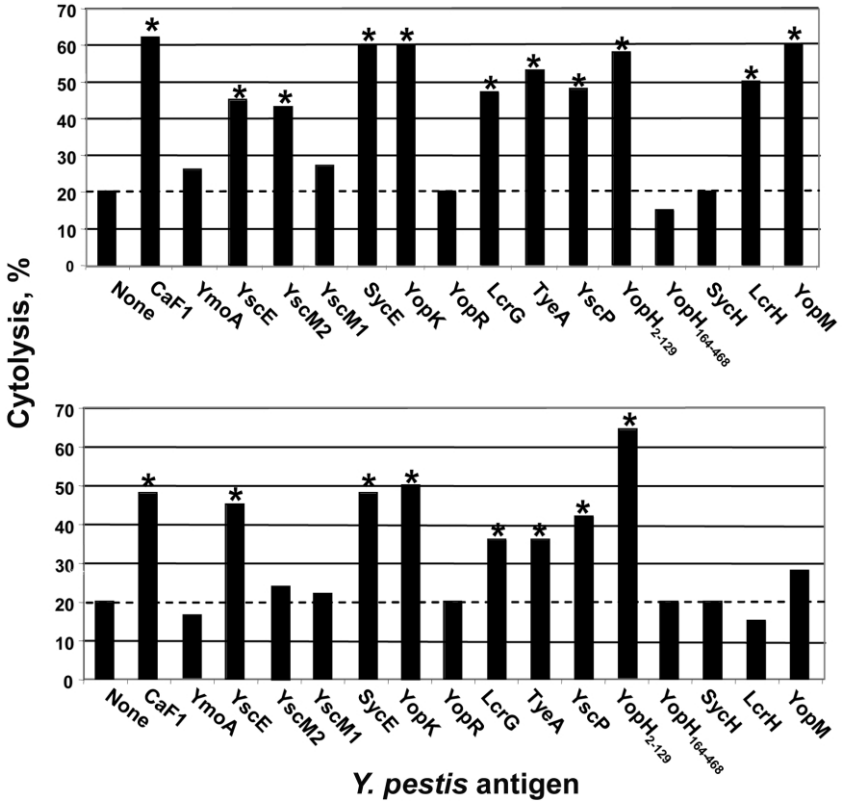

Figure 4. Cytotoxic T lymphocyte recognition of pCD1-encoded proteins presented by *Yersinia pestis*–infected monocytes *(top)* or dendritic cells *(bottom)*. Lysis of homozygous HLA-A2 B lymphoblasts sensitized by incubation (for 1 h at 37°C) with recombinant *Y. pestis* proteins (10 μg/mL) was assessed with T cells from infected cultures, and results were statistically significant ($P < .05$, analysis of variance with Dunnett's post hoc test) as indicated by asterisks. Data shown are from a representative experiment (total of 3 experiments, separate donors) and an optimal cell ratio of 1:10 (1:1 and 1:10, and 1:20 of BSMs:T cells). The fraction 1 capsular (CaF1) antigen was included as a positive control.

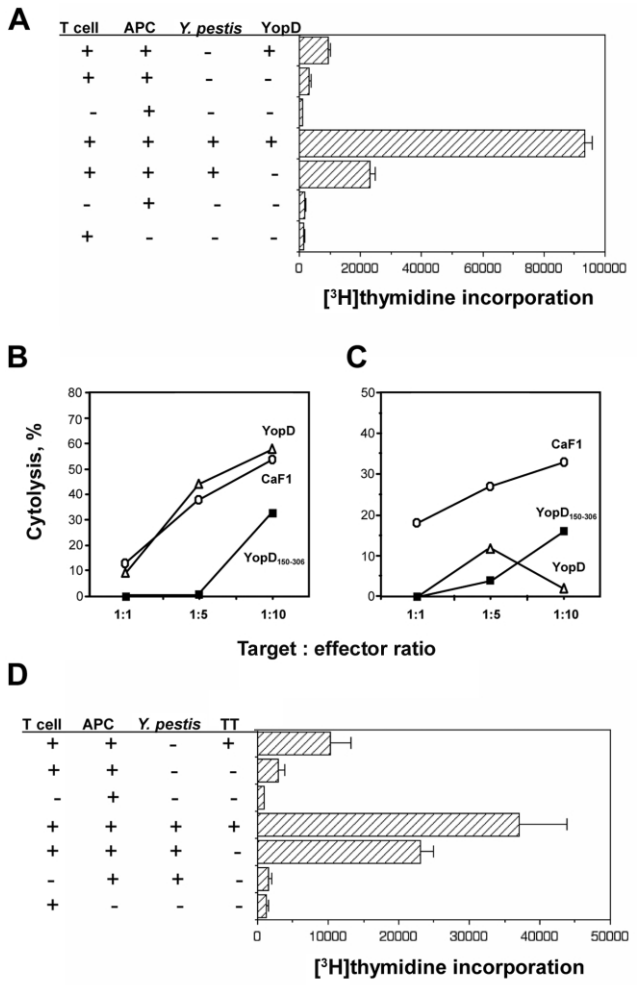

Figure 5. Primary T cell responses to the *Yersinia pestis* hydrophobic translocator protein (Yop) D. *A,* T cell proliferation in response to YopD (1 μg/mL) presented by *Y. pestis*–infected monocytes. The T cell response to YopD presented by infected monocytes, compared with that of uninfected controls, was significant ($P < .0001$). *B,* Cytotoxic T lymphocyte (CTL) recognition of purified YopD (1 μg/mL) presented by monocytes and *(C)* dendritic cells infected with *Y. pestis* by lysis of target cells. Data shown are results after the subtraction of background lysis of target cells without antigen (10%, 14%, and 20%) for monocyte-activated CTLs and (7%, 10%, and 18%) for CTLs activated by dendritic cells, corresponding to target:effector ratios of 1:1, 1:5, and 1:10, respectively. *D,* Presentation of heterologous antigens by infected monocytes (10 *Y. pestis*/cell). The response of autologous T cells to tetanus toxoid (TT) (52-kDa carboxy-terminal peptide) was statistically significant ($P < .02$, 2-tailed Student's *t* test), compared with cultures (T cell and antigen-presenting cells [APC]) without TT.

scribed elsewhere [10–13], was also observed. Because YscM2, although it is similar to YscM1, is expressed by *Y. enterocolitica* but not by *Y. pestis,* CTL recognition may involve a cross-reactive epitope. A similar cluster of antigens, with the exception of YscM2, YopM, and LcrH, was recognized by CTLs that were generated in culture with *Y. pestis*–infected DCs (figure 4, *bottom*). Internalization of *Y. pestis* bacilli by both monocytes and DCs was confirmed by confocal microscopy and flow cytometry (data not shown). It was also noted that, although the chaperone-binding portion of YopH contained within $YopH_{2–129}$was recognized by CTLs, neither the chaperone SycH nor the catalytic domain of YopH (164–468 aa) elicited a CTL response. Collectively, these results indicated that human CTL primed by infected monocytes or DCs lysed target cells pulsed with soluble proteins encoded by pCD1.

Primary T cell responses to the hydrophobic translocator protein YopD. All of the recombinant proteins studied up to this point were stable in solution. However, some pCD1-encoded factors might insert into cell membranes and are difficult to purify because of their high hydrophobicity. Because the hydrophobic protein YopD was previously shown to be partially protective in mice against lethal subcutaneous challenge with nonencapsulated *Y. pestis* C12 [19], we examined T cell responses to YopD presented by infected cells. Although the recombinant protein tended to precipitate in the cell-culture medium, significant T cell proliferative responses were observed with YopD presented by infected monocytes (figure 5*A*), and target cells sensitized with YopD were lysed by T cells activated with infected monocytes (figure 5*B*) or DCs (figure 5*C*). CTLs appeared to recognize YopD-pulsed target cells better if they had been primed by infected monocytes, compared with DCs (figure 5, 5*B* vs. 5*C*). In addition, lower responses to the more soluble carboxy-terminal fragment of YopD, compared with the full-length protein (figure 5), suggested that CTL epitopes were contained within both the amino and carboxyl termini.

Presentation of heterologous antigens by infected monocytes. Although infected monocytes presented *Y. pestis* proteins to T cells, there was the possibility that presentation of other proteins by these infected APCs may have been diminished. We examined this issue by measuring T cell responses to tetanus toxoid, a common recall antigen. Monocytes were infected with *Y. pestis,* followed by culture with the carboxy-terminal polypeptide of tetanus toxin and autologous T cells. Our results indicated that T cells readily proliferated in response to the tetanus toxin polypeptide presented by monocytes infected with *Y. pestis* (figure 5*D*).

DISCUSSION

Our results demonstrate that infected monocytes and DCs stimulate naive T cells to respond to many TTSS-associated virulence factors, including those that disrupt distinct host biochemical pathways. Significant CTL recognition of CaF1, YscM2, YscE, YopH, SycE, YopK, YopM, LcrG, TyeA, YscP, and LcrH was observed. Proliferative T cell responses were also noted to CaF1 [28], YopH, YopK (data not shown), and YopD presented by infected cells, which suggests that these antigens may be useful for inducing both CTL and T helper immunity. Fur-

thermore, adaptive T cell immunity is relevant to the earliest phase of plague, because the expression of the TTSS proteins is activated by transition of the host temperature to 37°C, which occurs shortly after transfer from the flea vector [33]. Although TTSS factors injected into the cytosol potentially attack both innate and adaptive arms of the immune response, infected human monocytes differentiate into DCs and appear to retain many normal functions [28].

It is perhaps surprising that T cell antigen receptors respond to potent virulence factors from *Y. pestis,* because the biochemical activities of these factors perturb host cell function. For example, YopH is a potent tyrosine phosphatase that attacks macrophage p130Cas, FAK proteins, and other tyrosine-phosphorylated proteins, which results in the disruption of focal adhesions [34, 35]; YopE is a GTPase-activating protein that causes actin cytoskeleton depolymerization [36]; and YpkA is a Ser/Thr kinase that interferes with P-mediated cellular signaling [37]. Although no specific enzymatic or other distinct biochemical activities were noted for YopM, this factor appears to target NK cells [38], which depletes an important host defense against intracellular infection. YopK is involved in regulating the translocation of Yops through membrane channels [39], and TyeA is required for the translocation of YopE and YopH [40]. In addition, the Ysc proteins [41] assemble into the injectosome, a macromolecular delivery system that directs the translocation of YopE, YopH, YopJ, YopM, YopT, and YpkA into the cytosol of mammalian cells [3].

Antibody responses play an important role in protective immunity to *Y. pestis.* Efforts to develop vaccines against *Y. pestis* infection have primarily focused on CaF1 and V (LcrV) antigens and a proposed antibody-mediated mode of immune protection [13, 42]. LcrV is a secreted protein encoded by pCD1 that regulates Yop translocation into host cells and may suppress immune responses by a mechanism involving IL-10 [43]. Antibody responses to YopH, YopD, and YopM were also detected in mice challenged with pneumonic plague and rescued by antibiotic treatment [18]. CTLs and phagocytes may also be critical to bacterial clearance because of the protected intracellular growth of *Y. pestis* at the earliest stage of infection. The intracellular expression of YopH [44] or CaF1 [20] was previously shown to stimulate adaptive mouse CTL immunity, although the contribution to bacterial clearance is unclear.

Although all stages of plague progression are potential therapeutic targets, the earliest medical intervention will be the most effective. Phagocytosis by neutrophils, macrophages, and certain other cells; inflammatory cytokine responses triggered by pathogen-associated molecular patterns; and NK cell activity may all function in the naive host to clear invading pathogens. Manufacturing of the US-licensed, formaldehyde-killed, whole bacterial vaccine was discontinued in 1999, and there are no other vaccines available at present for plague. A proposed vaccine comprised of recombinant CaF1 and LcrV components [10–13] was designed to primarily stimulate antibody responses. Circulating antibodies from vaccination or prior immunity may inhibit the delivery of *Y. pestis* virulence factors by promoting phagocytosis [45]. Because the intracellular phase sequesters *Y. pestis* from antibody contact, CTL activity and enhanced innate responses may be important for achieving sterilizing immunity, particularly in the naive host. Our results suggest a potential benefit of vaccination strategies that emphasize CTL responses, and we have identified several pCD1-derived proteins that are potential new vaccine candidates. Furthermore, we propose that the enhancement of CTL activity by therapeutic vaccination may also be useful for the treatment of infection.

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