Suppression of T and B Lymphocyte Activation by a Yersinia pseudotuberculosis Virulence Factor, YopH

By Tony Yao,* Joan Mecsas,† James I. Healy,* Stanley Falkow,‡ and Yueh-hsiu Chien*‡

From the *Program in Immunology and the †Department of Microbiology and Immunology, Stanford University, Stanford, California 94305

Summary

The acquired immune responses are crucial to the survival of Yersinia-infected animals. Mice lacking T cells are sensitive to Yersinia infection, and a humoral response to Yersinia can be protective. Diverse mechanisms for Yersinia to impair and evade the host innate immune defense have been suggested, but the effects of Yersinia on lymphocytes are not known. Here, we demonstrate that after a transient exposure to Y. pseudotuberculosis, T and B cells are impaired in their ability to be activated through their antigen receptors. T cells are inhibited in their ability to produce cytokines, and B cells are unable to upregulate surface expression of the costimulatory molecule, B7.2, in response to antigenic stimulation. The block of lymphocyte activation results from the inhibition of early phosphorylation events of the antigen receptor signaling complex. Through the use of Y. pseudotuberculosis mutants, we show that the inhibitory effect in both T cells and B cells is dependent on the production of Yersinia outer membrane protein (Yop) H, a tyrosine phosphatase. Our results suggest a mechanism by which the pathogenic bacteria may modulate a wide range of T and B cell–mediated immune responses.

Key words: T cell • B cell • Yersinia pseudotuberculosis • bacterial pathogenesis • YopH

An animal host must possess a well-developed adaptive immune response as well as robust innate immunity to clear most microbial pathogens. Pathogenic bacteria employ diverse mechanisms to avoid the innate immune defense system (1, 2). Several bacterial pathogens reprogram the course of endosomal development to prevent fusion with lysosomal elements, creating a privileged niche within host cells. Many bacteria produce surface components to resist phagocytosis and complement killing, while others possess enzymes that inactivate components of the host defense system. Known mechanisms by which bacteria affect the adaptive immune response are sparse. Mycobacterium tuberculosis has been reported to inhibit antigen presentation by macrophages through an unknown mechanism (3). Clearly, the adaptive immune response remains essential for host defense of bacterial pathogens, including the pathogenic members of the genus Yersinia (4, 5).

The genus Yersinia includes the causative agent of bubonic plague, Y. pestis, as well as the common enteric pathogens, Y. enterocolitica and Y. pseudotuberculosis. Infection with Y. pseudotuberculosis and Y. enterocolitica generally causes gastroenteritis and lymphadenitis. However, in some susceptible individuals, infection is associated with the development of reactive arthritis (6, 7). All three Yersinia species have a tropism for lymphatic tissue. In animal model systems, Y. pseudotuberculosis and Y. enterocolitica reach the intestinal tract, enter through the M cells of the Peyer's patches, and encounter the host cellular elements (8). Several days after infection, Yersinia are found in the mesenteric lymph nodes and, subsequently, in the spleen and liver. Unlike most bacterial enteropathogens that breach the epithelial barrier, such as Shigella and Salmonella species, Yersinia are not found intracellularly but, rather, are seen firmly fixed to the host cell surface (9).

An essential key to Yersinia's pathogenicity is the presence of a 70-kb plasmid (pYV) (10, 11). This plasmid, found in all three pathogenic Yersinia species, encodes a type III secretion system and several temperature- and calcium-regulated virulence proteins or effector molecules, the Yersinia outer membrane proteins (Yops).1 Yersinia also express several adhesion molecules that allow the bacteria to preferentially attach to different classes of integrin receptors on the surface of epithelial, fibroblast, macrophage, and lymphocyte cell lines. Direct cell contact, in combination with the type III secretion machinery, allows Yersinia to translocate the Yops from the cytoplasm of the bacteria directly into the cytoplasm of the host cell. Yops can be transported into the cytoplasm of the host cell via translocation proteins YopA and YopD (12, 13).

*Abbreviations used in this paper: BCR, B cell antigen receptor; FA, focal adhesion; HEL, hen egg lysozyme; MCC, moth cytochrome c; MOI, multiplicity of infection; Yop, Yersinia outer membrane protein.

J. Mecsas and J.I. Healy contributed equally to this work.
the cytoplasm of a host cell to modify host cell function(s). At least six virulence proteins are thought to be active in the host cell: YopH, YopE, YopJ/P, YopO, YopM, and YopT. The effects of these Yops have been studied primarily in macrophages and epithelial cell lines (10, 11). YopH, a tyrosine phosphatase, was reported to dephosphorylate p130Cas and paxillin, all tyrosine-phosphorylated proteins found in the focal adhesion (FA) complexes. YopH activity was found to cause the disassembly of FA, which impairs the entry of the bacteria into HeLa cells or their phagocytosis by macrophages (12–14). Additionally, YopH may function cooperatively with YopE in inhibiting phagocytosis by neutrophils (15). YopE activity is associated with depolymerization of the host cell cytoskeleton, thus preventing ingestion of the bacteria (16). YopJ/P, in certain conditions, induces apoptosis in macrophages in vitro and in vivo (17–19).

In other conditions, YopJ/P can affect nuclear factor κB-mediated signal transduction in macrophages through an unknown mechanism and, subsequently, inhibit TNF-α production (20–22). Although the functions of YopO, which has homology to serine/threonine kinases (23), and YopT are not clear, it has been shown that both YopO and YopT can perturb the cytoskeleton of epithelial cells in the absence of YopE (24, 25). YopM has homology to von Willebrand factor, suggesting that it may play a role in plasma clotting (26, 27). Thus, Yersinia produce a spectrum of virulence factors which when injected into host cells may alter host cell antiphagocytic function and, sometimes, host cell viability.

Although these observations suggest possible mechanisms for Yersinia to impair the host innate immune system, the effect of Yersinia on components of the adaptive immune system is not clear. This is despite the fact that acquired immune responses are crucial to the survival of infected animals and the observed link between Yersinia infection and autoimmunity. During the natural course of Yersinia infection, the bacteria almost certainly encounter lymphocytes as they colonize and multiply extracellularly in Peyer’s patches, mesenteric lymph nodes, spleen, and liver (5, 8). Indeed, T cells as well as macrophages are present in Yersinia-induced lesions in the spleen and liver (28). In vitro studies indicate that Yersinia can bind both T and B lymphocytes, presumably via the integrins on the lymphocytes (29, 30). Thus, T and B lymphocytes are potential targets for the bacteria in vivo.

In this study, we found that Y. pseudotuberculosis can directly interfere with T and B cell antigen receptor-mediated activation and that the lymphocyte inhibitory effects are dependent on the production of YopH, the tyrosine phosphatase. The presence of YopH in T and B cells results in hyperphosphorylation of almost all tyrosine-phosphorylated components associated with the antigen receptor signaling complex after receptor activation. Consequently, T cells, transiently exposed to Yersinia, were unable to flux calcium and produce cytokines. Likewise, primary B cells, transiently exposed to Y. pseudotuberculosis, were unable to up-regulate the costimulatory molecule, B7.2, in response to antigen stimulation. As a result, a wide range of T and B cell-mediated immune responses may be profoundly affected during infection. These observations suggest a novel way by which Yersinia can incapacitate the host adaptive immune response. Thus, Yersinia appears to produce effectors that are specifically designed for the different cell types that the bacteria encounter in the course of an infection.
B cells were isolated from MDA anti-hen egg lysozyme (HEL) Ig transgenic mouse spleens by negative depletions with anti-Thy1, anti-CD4, anti-CD8, and anti-Mac-1 (Caltag) as described (37). Cells were >98% B220 as assessed by FACS. Anti-HEL B cells were exposed to wild-type or mutant Y. pseudotuberculosis at an MOI of 50 for 1 h at 37°C, followed by the addition of HEL protein (500 ng/ml). After 3, 10, and 30 min, the B cells were lysed with cytoplasmic extract buffer, nuclei were removed by centrifugation, and 1 x 10^6 cell equivalents were diluted into 2 x reducing Laemmli buffer and run on 10% SDS-PAGE. Phosphotyrosine proteins were detected by Western blot as described above.

For B7.2 upregulation, freshly isolated anti-HEL Ig B cells were first incubated at 37°C with the specified Y. pseudotuberculosis at an MOI of 20. After 1 h, gentamicin was added to a final concentration of 100 µg/ml. Splenic B cells from anti-HEL Ig transgenic or C57BL/6 mice were stimulated with HEL (500 ng/ml) or anti-IgM (3 µg/ml; Jackson ImmunoResearch Laboratories), respectively, for 12 h at 37°C. B cells were then stained for FACS analysis using anti-B220–FITC (RA3-6B2; PharMingen) and anti-B7.2–PE (GL1; PharMingen). Propidium iodide was included to assess cell viability.

Results

Wild-type Y. pseudotuberculosis Inhibits Antigen-specific T Cell Activation in a YopH-dependent Manner. The development of a specific immune response is essential to resolve Yersiniosis. Since T cells are integral components of the specific immune response, we investigated whether Yersinia infection could significantly alter T cell activity by monitoring their cytokine production in response to antigenic activation. In the initial experiment, we preexposed either the APC, CH27, an I-E^k–expressing B cell lymphoma, or MCC9, an MCC/I-E^k specific T cell hybridoma, to either wild-type Yersinia or a Yersinia lcr mutant. The lcr mutant possesses the virulence plasmid, pYV, but is unable to produce or secrete Yops due to a mutation in a Yop regulatory locus (38, 39). After a 1-h incubation with Yersinia at an MOI of 10, the cells were treated with gentamicin (to kill the bacteria) and then mixed with MCC peptide and the appropriate untreated cells to achieve antigen activation. As shown in Fig. 1, in both cases, IL-2 production was significantly decreased after exposure to wild-type Yersinia but not to the lcr Yersinia mutant. This indicates that one or more secreted Yop(s) is responsible for the observed cytokine inhibition. Interestingly, the Yersinia inhibitory effect on cytokine production is much more pronounced when T cells were preexposed to Yersinia, suggesting that Yersinia's effect is greater on T cells than on CH27 cells. However, to definitively test if the bacteria were directly affecting T cells, we needed to exclude the possibility that, when the two cell types are mixed together, the bacteria interact with the other cell type before gentamicin killing takes effect. Therefore, plate-bound I-E^k/MCC peptide (88-103) protein complex, instead of CH27 cells, was used in subsequent T cell activation assays. MCC9 T cells exposed to wild-type, but not a virulence plasmid-deficient (pY^-V^-) or an lcr mutant (data not shown) Yersinia, showed a dramatic reduction in IL-2 production compared with unexposed controls (Fig. 1 C). These data suggest that one or more components of the Y. pseudotuberculosis virulence plasmid can directly inhibit T cell activation.

To determine which Yop(s) are necessary for the suppression of IL-2 production, several Yersinia Yop mutants were tested...
were screened in the T cell activation assay (Fig. 1 C). YopO- and YopE-deficient bacteria, but not YopH-deficient bacteria, were able to inhibit IL-2 production as efficiently as wild-type bacteria. In addition, the YopH mutant was unable to inhibit IL-2 production when MCC9 was stimulated by APCs (Fig. 1, A and B). These results suggest that YopH is necessary for the inhibition of cytokine production in T cells. Similarly, exposure to wild-type, but not pYV-cured or YopH-deficient mutant, 

\textit{Yersinia} inhibited the MCC/I-Ek-specific production of IL-3, IL-4, and IFN-\gamma by T cell lines derived from the SC.C7 TCR transgenic mice (data not shown). Cell viability, as assayed by propidium iodide staining and trypan blue exclusion, did not significantly change as a result of exposure to 

\textit{Yersinia} in any of the above experiments (data not shown). These data suggest that YopH may target a common component in cytokine activation pathways.

The YopH Inhibitory Effect Is Upstream of Calcium Flux and Protein Kinase C Activation. YopH has tyrosine phosphatase activity, and T cell activation through the antigen receptor is known to require the rapid phosphorylation of the tyrosine kinases, CD3\(\zeta\) chain, and linker/adaptor molecules (40). The phosphorylation and clustering of these molecules are critical to T cell activation, and these events precede the activation of protein kinase C as well as the rapid changes in intracellular calcium concentration accompanying T cell activation. Protein kinase C activation and the intracellular calcium flux are, in turn, required for induction of cytokine production. As shown in Fig. 2, the YopH inhibitory effect can be reversed by the addition of PM A/ionomycin, indicating that the YopH-mediated cytokine suppression was upstream of protein kinase C activation and calcium flux. Consistent with this supposition, the ability of 

\textit{Yersinia}-exposed SC.C7 T cells to flux calcium in response to antigen was impaired in a YopH-dependent manner (data not shown). These results suggest that the effect of YopH is largely limited to early tyrosine phosphorylation events in T cell signaling.

**Figure 2.** The inhibitory effect on T cell activation can be overridden by PM A and ionomycin. IL-2 production from MCC9 T cells exposed to wild-type (Wt), pYV\(-\) (P\(-\)), or YopH\(-\) (H\(-\)) 

\textit{Yersinia}, or medium alone (T) for 1 h and incubated overnight with PM A and ionomycin (PM A/Iono) or 60 \(\mu\)g/ml of plate-bound MCC/1-E\(\kappa\).

\textbf{YopH Inhibits the T Cell Tyrosine Phosphorylation Signal Cascade.} The biochemistry of antigen receptor signaling has been well characterized in the human T cell Jurkat line (for a review, see reference 40). Hence, we evaluated whether the induction of the tyrosine phosphorylation cascade in response to TCR cross-linking by the mAb OKT3 is altered when Jurkat cells are exposed to 

\textit{Yersinia}. As shown in Fig. 3, many tyrosine-phosphorylated proteins induced after TCR cross-linking, including CD3\(\zeta\), were severely reduced or absent in T cells exposed to wild-type or YopE-deficient, but not pYV\(-\) or YopH-deficient, mutant 

\textit{Yersinia} compared with the sham-exposed T cells. These results suggest that YopH targets some of the earliest initiators of the T cell signaling complex.

\textbf{YopH Inhibits B Cell Antigen-specific Responses.} The antigen receptor on B lymphocytes (BCR) consists of the membrane-bound Ig and associated Ig\(\alpha\) and Ig\(\beta\) molecules. Tyrosine phosphorylation of Ig\(\alpha\) and Ig\(\beta\) is required for the initiation of the tyrosine phosphorylation signaling cascade after antigen binding (41). Because of the signaling similarities between T cells and B cells, we tested whether the initial signaling events after BCR stimulation are affected by exposure to 

\textit{Y. pseudotuberculosis}. As in T cells, we found that exposure to wild-type 

\textit{Yersinia} interfered with the induction of the early tyrosine phosphorylation signaling cascade in response to antigen receptor engagement in a YopH-dependent manner (Fig. 4). Anti-HEL Ig transgenic B cells

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2}
\caption{Figure 2. The inhibitory effect on T cell activation can be overridden by PM A and ionomycin. IL-2 production from MCC9 T cells exposed to wild-type (Wt), pYV\(-\) (P\(-\)), or YopH\(-\) (H\(-\)) 

\textit{Yersinia}, or medium alone (T) for 1 h and incubated overnight with PM A and ionomycin (PM A/Iono) or 60 \(\mu\)g/ml of plate-bound MCC/1-E\(\kappa\).}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3}
\caption{Figure 3. YopH inhibits tyrosine phosphorylation of the TCR signaling complex. (A) Antiphosphotyrosine blot of resting (\textminus) and OKT3-activated (+) T cell lysates from Jurkat T cells exposed to either wild-type (Wt), YopE\(-\) (AE), YopH\(-\) (AH), or pYV\(-\) (P\(-\)) Y. pseudotuberculosis. Arrows indicate the heavy and light chains of OKT3. (B) Blots were stripped and reprobed for actin. (C) Antiphosphotyrosine blot of immunoprecipitated CD3\(\zeta\) from extracts.}
\end{figure}
were exposed to wild-type, pYV−, or Y opH-deficient Yersinia and activated with HEL. B cells exposed to wild-type but not pYV− or Y opH-deficient Yersinia showed a drastic reduction in the surface expression of B7.2 (Fig. 5) and CD69 (data not shown). Similarly, the induction of B7.2 and CD69 on splenic B cells isolated from C57BL/6 mice in response to anti-IgM cross-linking was also impaired when the B cells were exposed to wild-type but not pYV− or Y opH-deficient Yersinia (data not shown). This effect was not due to Yersinia-induced cell death since the number of live cells, as assessed by propidium iodide, was not significantly different among samples (data not shown).

Discussion

In this study, we have examined the interaction between Yersinia and the adaptive immune system by monitoring the response of T and B lymphocytes to antigen after a brief exposure to Yersinia. Our results provide evidence that the Y. pseudotuberculosis tyrosine phosphatase, Y opH, inhibits the signaling cascades associated with T and B cell antigen receptor activation. This is one of the first examples of a bacterial pathogen directly manipulating lymphocyte signaling and activation. Continuation of this inhibitory effect may explain why Yersinia infection, in some cases, is characterized by a chronic infection of lymphatic organs.

The consequences of the Y opH dephosphorylation activity may affect a wide range of T and B cell–mediated immune responses, including cytokine production. In fact, Yersinia infection dramatically suppresses the induction of IFN−γ and TNF−α production in some mouse strains (42, 43). When these cytokines are replaced exogenously, the mice are better able to survive infection. Since infection with an lcr− Yersinia triggers IFN−γ and TNF−α production in infected mice, one or more Yops are likely responsible for the suppression of cytokines. Recent experiments suggest that Y opP suppresses TNF−α production in macrophages (20–22), whereas we found that IFN−γ production was reduced in T cells infected with Yersinia. Thus, the inhibition in cytokine production observed after infection with wild-type Yersinia may be due to the combined action of Y opH and Y opP on different cell types. The pathological significance of our finding is not limited to a bacteria-induced suppression of cytokine production. The development of a T cell–mediated autoimmune disease, reactive arthritis, has long been associated with Y. pseudotuberculosis and Y. enterocolitica infection in genetically susceptible individuals. Inappropriate T cell signaling has been postulated as a factor in the development of autoimmune diseases. Thus, T cells exposed even briefly to wild-type Y. pseudotuberculosis may signal abnormally, if at all, for a significant period of time. If a stimulus is delivered to the TCR during a partially responsive period, the T cell may respond inappropriately, leading to the development of abnormal immune responses. In addition, complete T cell activation requires a signal delivered through TCR–peptide/MHC complex and the engagement of costimulatory molecules such as CD28/B7.2 complex and the engagement of costimulatory molecules such as CD28/B7.2.

Figure 4. Yersinia inhibits tyrosine phosphorylation of the BCR signaling complex in a Y opH–dependent manner. (A) Antiphosphotyrosine blot of cell extracts from splenic B cells from anti-HEL transgenic mice. B cells were incubated with medium alone (B), wild-type (WT), Y opH− (ΔH), or pYV− (P−) Yersinia for 1 h, then activated with HEL protein (500 ng/ml) for the indicated times. (B) Blots were stripped and reprobed for actin.

Figure 5. Y opH suppresses B cells antigen-specific B7.2 upregulation. Splenic B cells from anti-HEL Ig transgenic mice were exposed to either wild-type (A), pYV− (B), or Y opH− (C) Y. pseudotuberculosis before the addition of HEL protein. After 12 h, cells were stained with FITC-conjugated anti-B220, PE-conjugated B7.2, or propidium iodide, and analyzed by FACS®. Only B220+ and propidium iodide-negative cells (live) were included in the analysis. Bold lines represent expression of B7.2 on Yersinia-exposed, HEL-activated cells. Thin lines represent B7.2 expression on unexposed, HEL-activated B cells. At least three independent experiments were conducted, and a representative plot is shown.
has been suggested that the catalytic domain of YopH at the COOH-terminal end of the molecule selectively targets tyrosine-phosphorylated sites that contain the D/E PY xXP motif (45). In addition, a domain in the NH2-terminal region of YopH may be required for the efficient recognition of substrates. This substrate-binding domain exhibits a ligand specificity that is similar to that of the Crk Src homology 2 domain (13). Although the target(s) of YopH in lymphocytes is not known, we observed that most of the tyrosine kinases and adapter/linker proteins that are normally phosphorylated after T and B cell antigen receptor stimulation were either not phosphorylated or rapidly dephosphorylated in the presence of YopH. The three known YopH targets identified in epithelial and Hela cells, paxillin, p125FAK, and p130cas (12–14), and its closely related homologue, p105casL, are also found in lymphocytes (46). Further, p105casL, associated with the cell membrane and clustered integrins, is known to be phosphorylated after TCR ligation (47). Thus, as p105 casL may be involved in both TCR and integrin signaling, it is tempting to speculate that, in addition to the former, YopH may also target one or more members of the known T cell signaling cascade, including protein tyrosine kinases such as ZAP70 and Syk and tyrosine-phosphorylated adapter/linker proteins such as linker for activation of T cells (LAT), B cell linker protein (BLNK), and SH2 domain-containing leukocyte protein of 76 kD (SLP-76), which all contain sequences similar to the optimum YopH target consensus sequence. It is possible that a rapid dephosphorylation of these proteins may destabilize the TCR complex, which may lead to its disassembly and exposure of most or all components of the TCR complex, including CD3ζ, to phosphatases. We are currently characterizing the cellular distribution and protein targets of YopH in lymphocytes to distinguish among these possibilities.

What Cells Are Targets of Yops during Infection? The Yops play a crucial role in Yersinia virulence. Most past experiments have focused on the effects of these Yops in epithelial cells and macrophages. Yet, our work clearly demonstrates the rapid and essential role of YopH in inhibiting T cell and B cell signaling. Importantly, the redistribution and reorientation of the cytoskeleton are necessary early events for lymphocyte activation (48). Yet, both YopE and YopO, which have been shown to disrupt actin filaments leading to alterations in the cytoskeleton of both phagocytes and epithelial cells (16, 49), have no demonstrable effect in our assays. Furthermore, although YopE/YopO induces apoptosis in macrophages (17, 19), neither primary B cells, T cell lines, or T cell hybridomas appear susceptible to this virulence factor. YopH activity has been shown to mediate several effects in epithelial cells as well as macrophages, including: inhibition of Fc receptor-mediated oxidative burst in macrophages (50), inhibition of neutrophil functions (51, 52), FA disassembly in epithelium cells, and inhibition of phagocytosis in conjunction with YopE. These effects are all very different from the one we report here. Thus, it appears that different Yops target different host cell types involved in defense against Yersinia infection. By the same token, the enteropathogenic Yersinia harbor several different adhesion molecules that mediate their attachment to host cells. There is experimental evidence to suggest that some of the adhesins are better adapted to deliver specific Yops to certain host cell types. Invasin, which targets β1 integrins (53, 54), is essential for Peyer's patch translocation across the epithelial barrier, but not as efficient for delivering Yops to macrophages. Perhaps the plasmid-mediated adhesin, YadA, suffices in delivering Yops into macrophages. Taken together, it appears that Yersinia have evolved to produce effectors that are specifically designed for the different cell types that the bacteria encounter in the course of an infection.

The study of pathogen–host interactions provides a fascinating insight into the strategies used by microbes to manipulate normal host functions to their own benefit. The Yops are part of a complex secretion–translocation apparatus that responds to host cell cues and results in the delivery of an array of proteins into the host cell cytoplasm. Homologues of the secretory arm of this complex, the type III secretion system, have been found in a growing number of pathogenic gram-negative microorganisms. It is likely that other pathogenic microorganisms secrete molecules analogous to Yops to alter host cell function and cause immune suppression or an alteration in the immune response to the benefit of the microbe. It is instructive to see, for example, that Salmonella species have two fully functional type III secretion systems, one involved in entry and the other involved in intracellular replication, that are essential for virulence. Moreover, one of these Salmonella type III secretory pathways delivers an effector protein, SptP/SptA, into the host cell cytoplasm. SptP/SptA has a COOH-terminal phosphatase domain similar to that found in YopH and an NH2-terminal domain that is homologous to the YopE cytotoxin (55, 56). Curiously, infection with Salmonella can also trigger reactive arthritis in humans. Similarly, Shigella and Chlamydia and other pathogens have chromosomal islands containing type III secretory and effector homologues, and these bacteria are likewise associated with the development of autoimmune disease subsequent to infection. Is this the result of an underlying common strategy employed by many bacteria to alter the host immune response? Regardless, studying the molecular mechanisms of bacterial infection and host immune responses will reveal additional subtleties of bacterial pathogenicity and provide new information about the development of immune response.

We thank Jay Boniface and Dan Lyons (Stanford University) for the soluble MCC/I-Ek complex, Christoph Wülfing for help with the calcium flux experiments, Richard Glynne for the induction of B7.2 and CD69.
on B cells in response to anti-IgM, Arthur Weiss (University of California, San Francisco, CA) for anti-CD3; antibody 6810.2, Pat Jones (Stanford University) for the 5C.C7 T cell line, Diane Mathis and Christoph Benoist (Institut de Genetique et Biologie Moleculaire et Cellulaire, Strasbourg, France) for the MCCC9 T cells; and Mark Davis, John Xu, Denise Manack, and Tim McDaniel for helpful discussions.

This work is supported by grants from the National Institutes of Health (to Y. Chien), the Medical Scientist Training Program of the National Institutes of Health (to T. Yao and J.I. Healy), the American Cancer Society (PF-4477 to J. Mecsas), and the Defense Advanced Research Projects Agency (to J. Mecsas and S. Falkow). The content of the information does not necessarily reflect the position or the policy of the Government, and no official endorsements should be inferred.

Address correspondence to Yueh-hsiu Chien, Department of Microbiology and Immunology, Fairchild Bldg. D 333, Stanford University, Stanford, CA 94305. Phone: 650-723-1078; Fax: 650-725-6757; E-mail: chien@leland.stanford.edu

Submitted: 28 July 1999 Accepted: 20 August 1999

References

1. Finlay, B., and P. Cosart. 1997. Exploitation of mammalian host cell functions by bacterial pathogens. Science. 276:718–725.

2. Sherris, J., and K. Ryan. 1994. Sherris Medical Microbiology. 3rd ed. K. Ryan, editor. Appleton & Lange, East Norwalk, CT. 890 pp.

3. Pancholi, P., A. Mirza, N. Bhardwaj, and R.M. Steinman. 1992. T lymphocytes mediate protection against Yersinia enterocolitica in mice: characterization of murine T-cell clones specific for Y. enterocolitica. Infect. Immun. 60:1140–1149.

4. Autenrieth, I.B., U. Vogel, S. Preger, B. Heymer, and J. Heesemann. 1993. Experimental Yersinia enterocolitica infection in euthymic and T-cell-deficient athymic nude C57BL/6 mice: comparison of time course, histomorphology, and immune response. Infect. Immun. 61:2585–2595.

5. Tchuikova, K., and V. Pokrovski. 1998. Clinical and immune peculiarities of pseudotuberculosis polyarthritis against a background of chronic opisthorchiasis. Br. J. Rheumatol. 37:341–342.

6. Toivanen, A., K. Granfors, R. Lahesmaa-Rantala, R. Leino, T. Stahlberg, and R. Vuento. 1985. Pathogenesis of Yersinia-triggered reactive arthritis: immunological, microbiological and clinical aspects. Immunol. Rev. 86:47–70.

7. Autenrieth, I.B., and R. Firsching. 1996. Penetration of M cells and destruction of Peyer's patches by Yersinia enterocolitica: an ultrastructural and histological study. J. Med. Microbiol. 44:285–294.

8. Simonet, M., S. Richard, and P. Berche. 1990. Electron microscopic evidence for in vivo extracellular localization of Yersinia pseudotuberculosis harboring the pYV plasmid. Infect. Immun. 58:841–845.

9. Cornelis, G.R., and H. Wolf-Watz. 1997. The Yersinia Yop virulon: a bacterial system for subverting eukaryotic cells. Mol. Microbiol. 23:861–867.

10. Cornelis, G.R., A. Boland, A.P. Boyd, C. Geuijen, M. Iriarte, C. Neyt, M.P. Sory, and I. Stainier. 1998. The virulence plasmid of Yersinia, an antithost genome. Mol. Biol. Rev. 62:1315–1352.

11. Person, C., N. Carabellea, H. Wolf-Watz, and M. Fallman. 1997. The PT Pase YopO inhibits uptake of Yersinia, tyrosine phosphorylation of p130C as and FAK, and the associated accumulation of these proteins in peripheral focal adhesions. EMBO (Eur. Mol. Biol. Organ.) J. 16:2307–2318.

12. Black, D.S., L.G. Montagna, S. Zitzmann, and J.B. Bliksa. 1998. Identification of an amino-terminal substrate-binding domain in the Yersinia tyrosine phosphatase that is required for efficient recognition of focal adhesion targets. Mol. Microbiol. 29:1263–1274.

13. Black, D.S., and J.B. Bliksa. 1997. Identification of p130C as a substrate of Yersinia YopH (Yop51), a bacterial protein tyrosine phosphatase that translocates into mammalian cells and targets focal adhesions. EMBO (Eur. Mol. Biol. Organ.) J. 16:2730–2744.

14. Monack, D.M., J. Mecsas, S. Ghori, and S. Falkow. 1997. Yersinia signals macrophages to undergo apoptosis and YopJ is necessary for this cell death. Proc. Natl. Acad. Sci. U.S.A. 94:10385–10390.

15. Monack, D.M., J. Mecsas, D. Bouley, and S. Falkow. 1998. Yersinia-induced apoptosis in vivo aids in the establishment of a systemic infection of mice. J. Exp. Med. 188:2127–2137.

16. Rosqvist, R., A. Forsberg, and H. Wolf-Watz. 1999. Intracellular targeting of the Yersinia YopE cytotoxin in mammalian cells induces actin microfilament disruption. Infect. Immun. 59:4562–4569.

17. Monack, D.M., J. Mecsas, N. Ghorii, and S. Falkow. 1997. Y. enterocolitica induces apoptosis to evade microbicidal action of neutrophils. Infect. Immun. 65:724–733.

18. Schesser, K., A.K. Spilk, J.M. Dukuzumuremyi, M.F. N'jouw, S. Pettersson, and H. Wolf-Watz. 1998. The yopJ locus is required for bacterial-induced macrophage apoptosis. Proc. Natl. Acad. Sci. U.S.A. 94:12638–12643.

19. Mets, J., J. Tchkem, J.M. Dukuzumuremyi, M.F. N'jouw, S. Pettersson, and H. Wolf-Watz. 1997. Yersinia pseudotuberculosis induces macrophages by a process requiring functional type III secretion and translocation mechanisms and involving YopP, presumably acting as an effector protein. Proc. Natl. Acad. Sci. U.S.A. 94:12638–12643.

20. Schesser, K., A.K. Spilk, J.M. Dukuzumuremyi, M.F. N'jouw, S. Pettersson, and H. Wolf-Watz. 1999. The yopJ locus is required for bacterial-mediated inhibition of NF-κB activation and cytokine expression: YopJ contains a eukaryotic SH2-like domain that is essential for its repressive activity. Mol. Microbiol. 28:1067–1079.

21. Boland, A., and G.R. Cornelis. 1998. Role of YopM in suppression of tumor necrosis factor alpha release by macrophages during Yersinia infection. Infect. Immun. 66:1878–1884.

22. Palmer, L.E., S. Hobbie, J.E. Galan, and J.B. Bliksa. 1997. YopJ of Yersinia pseudotuberculosis is required for the inhibition of macrophage TNF-α production and downregulation of the MAP kinases p38 and JNK. Mol. Microbiol. 27:953–965.

23. Galyov, E.E., S. Hakansson, A. Forsberg, and H. Wolf-Watz. 1993. A secreted protein kinase of Yersinia pseudotuberculosis is an indispensable virulence determinant. Nature. 361:730–732.
24. Hakansson, S., E.E. Galyov, R. Rosqvist, and H. Wold-Watz. 1996. The Yersinia YopA Ser/Thr kinase is translocated and subsequently targeted to the inner surface of the HeLa cell plasma membrane. Mol. Microbiol. 20:593–603.

25. Iriarte, M., and G.R. Cornelis. 1998. YopT, a new Yersinia Yop effector protein, affects the cytoskeleton of host cells. Mol. Microbiol. 29:915–929.

26. Leung, K.Y., B.S. Reisner, and S.C. Slay. 1990. YopM inhibits platelet aggregation and is necessary for virulence of Yersinia pestis in mice. Infect. Immun. 58:3262–3271.

27. Leung, K.Y., and S.C. Slay. 1989. The yopM gene of Yersinia pestis encodes a released protein having homology with the human platelet surface protein GP Ib alpha. J. Bacteriol. 171:4623–4632.

28. Autenrieth, I.B., P. Hantschmann, B. Heymer, and J. Heese-mann. 1993. Immunohistological characterization of the cellular immune response against Yersinia pestis in mice: evidence for the involvement of T lymphocytes. Immunobiology. 187:1–16.

29. Lundgren, E., N. Carballeira, R. Vazquez, E. Dubinina, H. Branden, H. Persson, and H. Wold-Watz. 1996. Invasion of Yersinia pseudotuberculosis activates human peripheral B cells. Infect. Immun. 64:829–835.

30. Arencibia, I., N.C. Suarez, H. Wold-Watz, and K.G. Sundqvist. 1997. Yersinia pseudotuberculosis activates human peripheral B cells. Infect. Immun. 65:6497–6502.

32. Mecsas, J., B. Raupach, and S. Falkow. 1998. The Yersinia Yop inhibits invasion of Listeria, Shigella and Edwardsiella but not Salmonella into epithelial cells. Mol. Microbiol. 28:1269–1281.

36. van Oers, N.S., S.J. Teh, B.A. Irving, J. Tiong, A. Weiss, and H.S. Teh. 1994. Production and characterization of monoclonal antibodies specific for the murine T cell receptor zeta protein that is related to both CD28/B7 costimulation: a review. Crit. Rev. Immunol. 14:161–193.

37. Healy, J.I., R.E. Dolmetsch, L.A. Timmerman, J.G. Cyster, M.L. Thomas, G.R. Crabtree, R.S. Lewis, and C.C. Goodnow. 1997. Different nuclear signals are activated by the B cell receptor during positive versus negative signaling. Immunity. 6:419–428.

38. Sarker, M.R., M.P. Sory, A.P. Boyd, M. Iriarte, and G.R. Cornelis. 1998. LcrG is required for efficient translocation of Yersinia Yop effector proteins into eukaryotic cells. Infect. Immun. 66:2976–2979.

39. Bergman, T., S. Hakansson, A. Forsberg, L. Norlander, A. Macelaro, A. Backman, I. Bolin, and H. Wold-Watz. 1991. Analysis of the V antigen lcrGVH-yopBD operon of Yersinia pseudotuberculosis: evidence for a regulatory role of LcrH and LcrV. J. Bacteriol. 173:1607–1616.

40. Cantrell, D. 1996. T cell antigen receptor signal transduction pathways. Annu. Rev. Immunol. 14:259–274.

41. Reth, M., and J. Wienands. 1997. Initiation and processing of signals from the B cell antigen receptor. Annu. Rev. Immunol. 15:453–479.

42. Autenrieth, I.B., and J. Heesemann. 1992. In vivo neutralization of tumor necrosis factor-alpha and interferon-gamma abrogates resistance to Yersinia enterocolitica infection in mice. Med. Microbiol. Immunol. 181:333–338.

43. Naka-jima, R., and R.R. Brubaker. 1993. Association between virulence of Yersinia pestis and suppression of gamma interferon and tumor necrosis factor alpha. Infect. Immun. 61:23–31.

44. Greenfield, E.A., K.A. N guyen, and V.K. Kuchroo. 1998. CD28/B7 costimulation: a review. Crit. Rev. Immunol. 18:389–418.

45. Zhang, Z.Y., A.M. Thieme-Sefler, D. Maclean, D.J. M. Namara, E.M. Dobrusin, T.K. Sawyer, and J.E. Dixon. 1993. Substrate specificity of the protein tyrosine phosphatase ProCat. Nat. Acad. Sci. U.S.A. 90:4446–4450.

46. M. ingeshi, M., K. Tachibana, T. Sato, S. Iwata, Y. Nojima, and C. Morimoto. 1996. Structure and function of Cas-L, a 105-kD Crk-associated substrate-related protein that is involved in p1 integrin-mediated signaling in lymphocytes. J. Exp. Med. 184:1365–1375.

47. Kanda, H., T. Imamura, N. Morino, K. Hamaaki, T. Nakamoto, H. Hirai, C. Morimoto, Y. Yazaki, and Y. Nojima. 1991. Ligation of the T cell antigen receptor induces tyrosine phosphorylation of p105CasL, a member of the p130Cas-related docking protein family, and its subsequent binding to the Src homology 2 domain of c-Crk. Eur. J. Immunol. 27:2113–2117.

48. Monks, C.R., B.A. Freiberg, H. Kupfer, N. Slak, and A. Kupfer. 1998. T-cell compartmentalization of supramolecular activation clusters in T cells. Nature 395:82–96.

49. Holmstrom, A., R. Rosqvist, H. Wold-Watz, and A. Forsberg. 1995. Virulence plasmid-encoded YopK is essential for Yersinia pseudotuberculosis to cause systemic infection in mice. Infect. Immun. 63:2269–2276.

50. Bliska, J.B., and D.S. Black. 1995. Inhibition of the Fc receptor-mediated oxidative burst in macrophages by the Yersinia pseudotuberculosis tyrosine phosphatase. Infect. Immun. 63:681–685.

51. Wolkos, S., A. Friedlander, D. Mcdowell, J. Weeks, and S. Tobery. 1998. V antigen of Yersinia pestis inhibits neutrophil chemotaxis. J. Immunol. 155:3060–3066.

52. Anderson, K., K. M. M agnusson, M. Ajeed, O. Stendahl, and M. Fallman. 1999. Yersinia pseudotuberculosis-induced calcium signaling in neutrophils is blocked by the virulence effector YopH. Infect. Immun. 67:2567–2574.

53. Isberg, R.R., L. Voors, and H. Wolf-Watz. 1998. Identification of invasin: a protein that allows bacterial infection in mice. J. Exp. Med. 187:2113–2117.

54. Arricau, N., D. Hermant, H. Wakin, and M.Y. Popoff. 1997. Molecular characterization of the Salmonella typhimurium StpA protein that is related to both Yersinia YopE cytotoxin and YopH tyrosine phosphatase. Res. Microbiol. 148:21–26.

55. Kaniga, K., J. Uralil, J.B. Bliska, and J.E. Galan. 1996. A secreted protein tyrosine phosphatase with modular effector domains in the bacterial pathogen Salmonella typhimurium. Mol. Microbiol. 21:633–641.