

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

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## 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* outermembrane 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 tro-

pism 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* outermembrane proteins (Yops).<sup>1</sup> *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

<sup>1</sup>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* outermembrane protein.

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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 p130<sup>cas</sup>, p125<sup>FAK</sup>, 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  $\kappa$ B-mediated signal transduction in macrophages through an unknown mechanism and, subsequently, inhibit TNF- $\alpha$  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 hypophosphorylation 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 upregulate 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* seems to have evolved to produce effectors that are specifically designed for the different cell types that the bacteria encounter in the course of an infection.

## Materials and Methods

**Cell Lines, *Y. pseudotuberculosis* Strains, and Growth Conditions.** 5C.C7 T cell lines derived from 5C.C7 TCR transgenic mice (specific for moth cytochrome c [MCC]/I-E<sup>k</sup>; reference 31) were maintained in RPMI supplemented with 1-glutamine, nonessential amino acids, sodium pyruvate, penicillin/streptomycin, and 10% fetal bovine serum. T cell lines were stimulated every 2 wk with irradiated splenocytes and MCC peptide (88–103). 2 d after the addition of peptide and splenocytes, the medium was supplemented with 5 U/ml of IL-2. Jurkat T cells, MCC9 T cell hybridoma (specific for MCC/I-E<sup>k</sup> [32]), and CH27 were grown in the RPMI medium using 10% bovine calf serum. Primary B cell cultures were maintained in RPMI supplemented with 3% fetal bovine serum.

Wild-type and mutant *Y. pseudotuberculosis* (33) were initially grown overnight to plateau phase in 2  $\times$  YT at 26°C. Cells were diluted 1:50 into 2  $\times$  YT supplemented with 20 mM sodium oxalate and 20 mM magnesium chloride and grown for 2 h at 26°C, then shifted to 37°C for 2 h. Bacterial numbers were determined by OD<sub>600</sub> = 1  $\times$  10<sup>9</sup> bacteria/ml.

**Lymphocyte Infection and Activation.** MCC9 T cells, extensively washed with antibiotic-free medium, were exposed to the specified *Y. pseudotuberculosis* strain at a multiplicity of infection (MOI) of 10 for 1 h at 37°C. Gentamicin (final concentration 100  $\mu$ g/ml) was added to kill the bacteria followed by T cell activation assays. T cells in 96-well plates were activated by either MCC peptide (88–103) complexed with glycosyl phosphatidylinositol-linked I-E<sup>k</sup> (34), 1  $\times$  10<sup>5</sup> CH27 cells and MCC peptide, or 1  $\mu$ M ionomycin and 25 ng/ml PMA in 200  $\mu$ l of supplemented RPMI medium. After overnight incubation at 37°C, 100  $\mu$ l of medium was assayed for IL-2 production by standard sandwich ELISA (JES6-1A12, JES6-5H4; PharMingen) and streptavidin-europium detection (Wallac). For calcium flux, experiments were carried out by exposing 5C.C7 T cells to *Y. pseudotuberculosis* at an MOI of 50 for 1 h. Cells were loaded with the calcium-sensitive dye, Fura-2 (Molecular Probes), washed, and stimulated by an MCC peptide-loaded, confluent layer of I-E<sup>k</sup>-expressing Chinese hamster ovary cells, and monitored as described previously (35).

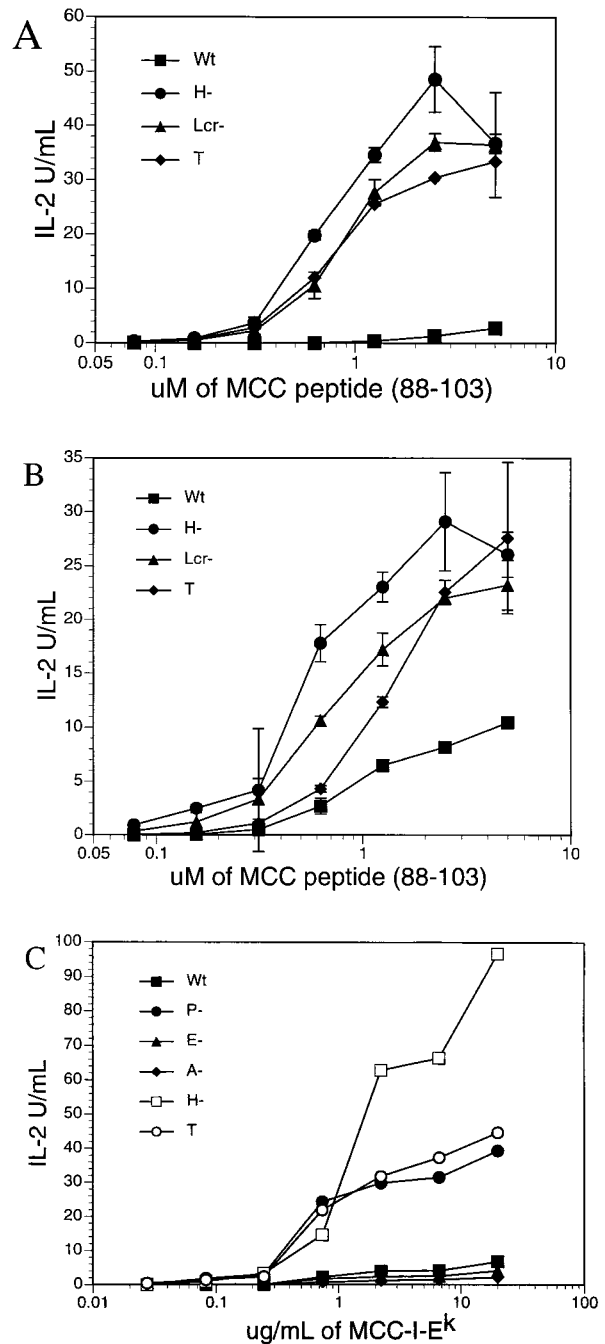
Jurkat T cells were exposed to specified *Y. pseudotuberculosis* for 1 h at an MOI of 50 at 37°C, followed by the addition of anti-CD3 (OKT3 ascites, 1:100). After 5 min, cells were spun down at 4°C and lysed with cytoplasmic extract buffer (1% NP-40, 150 mM NaCl, 10 mM Tris [pH 8.0], 2 mM sodium orthovanadate, 10 mM sodium fluoride, 5 mM  $\beta$ -glycerol phosphate, 5 mM dinitrophenyl phosphate, 100  $\mu$ g/ml leupeptin, 100  $\mu$ g/ml pepstatin, and 1  $\mu$ M PMSF). Nuclei were removed by centrifugation at 4°C. The cytoplasmic extract (1  $\times$  10<sup>6</sup> cell equivalents) was diluted into 2  $\times$  reducing Laemmli buffer and run on 10% SDS-PAGE. Phosphotyrosine proteins were detected by Western blotting, using the unconjugated antiphosphotyrosine antibody, 4G10 (Upstate Biotechnology), followed by an anti-mouse Ig-horseradish peroxidase conjugate (P0260; Dako) for detection. Blots were developed with enhanced chemiluminescence (Amersham Pharmacia Biotech). Each blot was subsequently stripped and reprobed for  $\beta$ -actin. CD3 $\zeta$  immunoprecipitations were conducted as described (36).

B cells were isolated from MD4 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<sup>+</sup> as assessed by FACS<sup>®</sup>. 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 × 10<sup>6</sup> cell equivalents were diluted into 2× 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<sup>®</sup> 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<sup>k</sup>-expressing B cell lymphoma, or MCC9, an MCC/I-E<sup>k</sup> 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) was 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<sup>k</sup>/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 (pYV<sup>-</sup>) 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



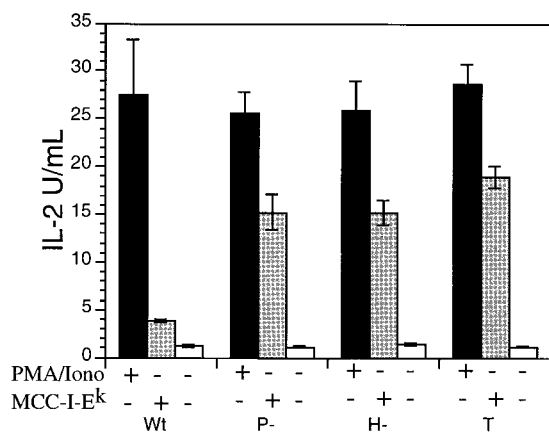
**Figure 1.** *Y. pseudotuberculosis* suppresses T cell antigen-specific IL-2 production in a YopH-dependent manner. IL-2 production of T cell activation assays with (A) MCC9 or (B) CH27 preexposed to either wild-type (Wt), YopH mutant (H<sup>-</sup>), Lcr mutant (Lcr<sup>-</sup>) *Yersinia*, or medium alone (T); or with (C) MCC9 T cells activated by plate-bound MCC/I-E<sup>k</sup> after the T cells were preexposed to wild-type (Wt), YopH mutant (H<sup>-</sup>), YopE mutant (E<sup>-</sup>), YopO/YopJ mutant (A<sup>-</sup>), pYV<sup>-</sup> (P<sup>-</sup>) *Yersinia*, or medium alone (T). At least three independent experiments were conducted, and representative result is shown.

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 screened in the T cell activation assay (Fig. 1 C). YopO/ YopJ- 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, *Yersinia* inhibited the MCC9/I-E<sup>k</sup>-specific production of IL-3, IL-4, and IFN- $\gamma$  by T cell lines derived from the 5C.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 *Yersinia* in any of the above experiments (data not shown). These data suggest that YopH may target a common component in cytokine activation pathways.

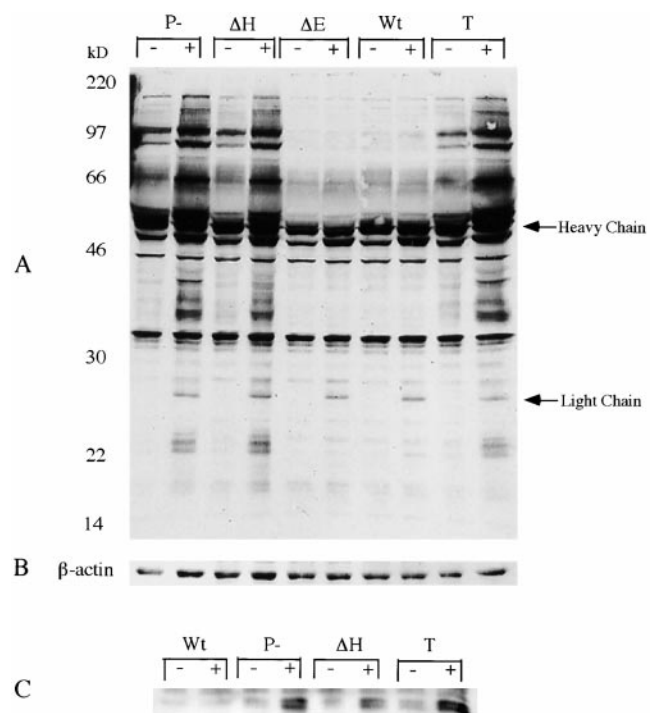
**The YopH Inhibitory Effect Is Upstream of Calcium Flux and Protein Kinase C Activation.** YopH has tyrosine phosphatase activity, and T cell activation through the antigen receptor is known to require the rapid phosphorylation of the tyrosine kinases, CD3 $\zeta$  chain, and linker/adaptor molecules (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 PMA/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 *Yersinia*-exposed 5C.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.



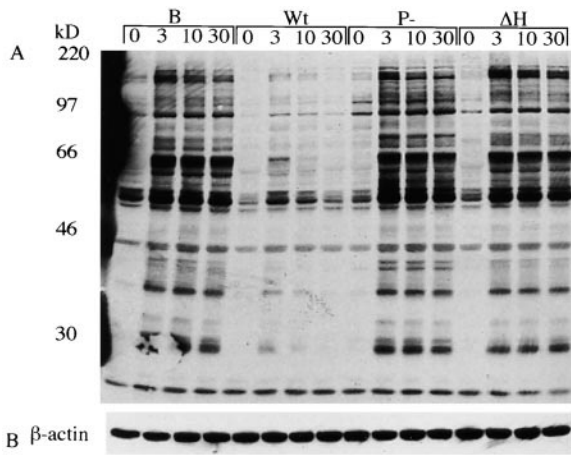
**Figure 2.** The inhibitory effect on T cell activation can be overridden by PMA and ionomycin. IL-2 production from MCC9 T cells exposed to wild-type (Wt), pYV<sup>-</sup> (P-), or YopH<sup>-</sup> (H-) *Yersinia*, or medium alone (T) for 1 h and incubated overnight with PMA and ionomycin (PMA/Iono) or 60  $\mu$ g/ml of plate-bound MCC9/I-E<sup>k</sup>.

**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 *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<sup>-</sup> or YopH-deficient, mutant *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.

**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 *Y. pseudotuberculosis*. As in T cells, we found that exposure to wild-type *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



**Figure 3.** YopH inhibits tyrosine phosphorylation of the TCR signaling complex. (A) Antiphosphotyrosine blot of resting (-) and OKT3-activated (+) T cell lysates from Jurkat T cells exposed to either wild-type (Wt), YopE<sup>-</sup> ( $\Delta$ E), YopH<sup>-</sup> ( $\Delta$ H), or pYV<sup>-</sup> (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.



**Figure 4.** *Yersinia* inhibits tyrosine phosphorylation of the BCR signaling complex in a YopH-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), YopH<sup>-</sup> ( $\Delta$ H), or pYV<sup>-</sup> (P<sup>-</sup>) *Yersinia* for 1 h, then activated with HEL protein (500 ng/ml) for the indicated times. (B) Blots were stripped and reprobed for actin.

were exposed to wild-type, pYV<sup>-</sup>, or YopH-deficient *Yersinia* and activated with HEL. B cells exposed to wild-type but not pYV<sup>-</sup> or YopH-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<sup>-</sup> or YopH-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

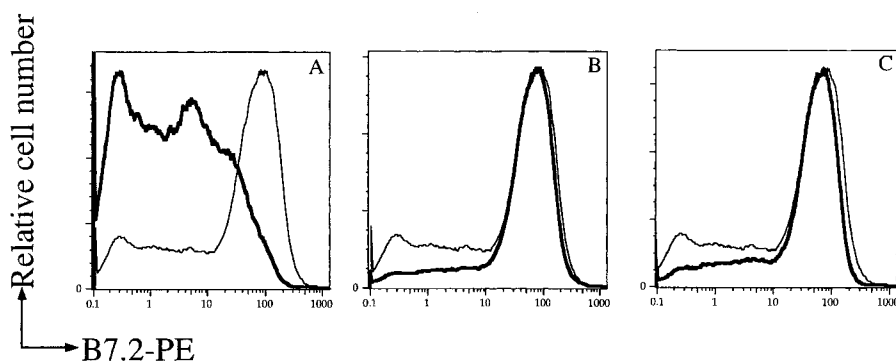
In this study, we have examined the interaction between *Yersinia* and the adaptive immune system by monitoring the response of T and B lymphocytes to antigen after a brief exposure to *Yersinia*. Our results provide evidence that the *Y. pseudotuberculosis* tyrosine phosphatase, YopH, inhibits the

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 YopH 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- $\gamma$  and TNF- $\alpha$  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 *la*<sup>-</sup> *Yersinia* triggers IFN- $\gamma$  and TNF- $\alpha$  production in infected mice, one or more Yops are likely responsible for the suppression of cytokines. Recent experiments suggest that YopJ/P suppresses TNF- $\alpha$  production in macrophages (20–22), whereas we found that IFN- $\gamma$  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 YopH and YopJ/P 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 aberrantly, 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. A failure to induce normal levels of B7.2 on APCs has been associated with the induction of T cell nonresponsiveness (44). Although many other factors are likely to be involved in the development of reactive arthritis, we believe that the ability of *Yersinia* to disrupt TCR and BCR signaling cascades may contribute to this condition in some individuals.

*What Proteins Are Target(s) of YopH in T and B Cells?* It



**Figure 5.** YopH 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<sup>-</sup> (B), or YopH<sup>-</sup> (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<sup>®</sup>. Only B220<sup>+</sup> 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/EpYxxP motif (45). In addition, a domain in the NH<sub>2</sub>-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, p125<sup>FAK</sup>, and p130<sup>cas</sup> (12–14), and its closely related homologue, p105<sup>casL</sup>, are also found in lymphocytes (46). Further, p105<sup>casL</sup>, associated with the cell membrane and clustered integrins, is known to be phosphorylated after TCR ligation (47). Thus, as p105<sup>casL</sup> may be involved in both TCR and integrin signaling, it is tempting to speculate that, in addition to the CD3 $\zeta$ -ZAP70 in T cells or the Ig $\alpha$ /Ig $\beta$  pathway in B cells, a second pathway involving paxillin-p125<sup>FAK</sup>-p105<sup>casL</sup> contributes to lymphocyte activation. An abrogation of p105<sup>casL</sup> phosphorylation may have a significant inhibitory effect on T cell function. Alternatively, YopH may also target one member or 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 CD3 $\zeta$ , 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 YopJ/YopP 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  $\beta$ 1 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 secretory 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 NH<sub>2</sub>-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.

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